## REMARKS

The Office Action mailed July 2, 2003, has been received and reviewed. Claims 17 through 27, 29 through 31, 33 through 41, and 49 through 53 are currently pending. Claims 17 through 27, 29 through 31, 36 through 38, 40 and 49 through 53 stand rejected. Claims 33 through 35, 39 and 41 have been objected to as being dependent upon rejected base claims, but the indication of allowable subject matter in such claims is noted with appreciation. Claims 17 through 27, 29 through 31, 33 through 41, and 49 through 53 have been amended herein. Applicants respectfully request reconsideration of the application in view of the above amendments and following remarks.

**Supplemental Information Disclosure Statement**

Please note that a Supplemental Information Disclosure Statement was filed herein on July 28, 2000, and that no copies of the forms PTO-1449 were returned with the outstanding Office Action. Applicants respectfully request that the information cited on the forms PTO-1449 be made of record herein. For the sake of convenience, a second copy of the July 28, 2000, Supplemental Information Disclosure Statement, forms PTO-1449 and USPTO date-stamped postcard are enclosed herewith. It is respectfully requested that an initialed copy of the forms PTO-1449 evidencing consideration of the cited references be returned to the undersigned attorney.

**35 U.S.C. § 112 Rejections**

Claims 50 and 51 stand rejected under 35 U.S.C. § 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which Applicants regard as the invention (*Office Action*, at page 2). In particular, claims 50 and 51 have been rejected due to an inadvertent inconsistency between the solvent recited in independent claim 17 and the solvents recited in claims 50 and 51. Each of claims 17, 50 and 51 have been amended herein to alleviate this inconsistency and it is respectfully submitted that the rejection has been overcome. As such, withdrawal of the 35 U.S.C. § 112 rejection of claims 50 and 51 is respectfully requested.

**35 U.S.C. § 102(b) Rejection**

A. Applicable Authority

A claim is anticipated only if each and every element as set forth in the claim is found, either expressly or inherently described, in a single prior art reference. *Verdegaal Brothers v. Union Oil Co. of California*, 2 USPQ2d 1051, 1053 (Fed. Cir. 1987). The identical invention must be shown in as complete detail as is contained in the claim. *Richardson v. Suzuki Motor Co.*, 9 USPQ2d 1913, 1920 (Fed. Cir. 1989).

B. 35 U.S.C. § 102(b) Rejection of Claims 17 through 27, 29 through 31, 36, 37, 52 and 53 Based Upon U.S. Patent 5,843,891

Claims 17 through 27, 29 through 31, 36, 37, 52 and 53 stand rejected under 35 U.S.C. § 102(b) as being anticipated by U.S. Patent 5,843,891 to Sherman (hereinafter the "Sherman reference") (*Office Action*, at page 3). As the Sherman reference fails to describe, either expressly or inherently, each and every element as set forth in the rejected claims, Applicants respectfully traverse this rejection, as hereinafter set forth.

For the sake of convenience, the independent claims to which the 35 U.S.C. § 102(b) rejection applies are summarized herein. Independent claim 17, as amended herein, recites a formulation comprising at least one beneficial agent and a non-aqueous, single-phase biocompatible vehicle. The vehicle comprises the solvent lauryl lactate, a surfactant, and a polymer. Each of the solvent, the surfactant and the polymer are selected and formulated such that the vehicle exhibits a viscosity capable of suspending the at least one beneficial agent.

Independent claim 18, as amended herein, recites a non-aqueous formulation comprising at least one beneficial agent uniformly suspended in a vehicle comprising a solvent, a surfactant, and a polymer, wherein the solvent is lauryl lactate and the solvent, surfactant and polymer are selected and formulated such that the vehicle is a non-aqueous, single-phase biocompatible vehicle that exhibits a viscosity capable of suspending the at least one beneficial agent.

Independent claim 36 recites a method for treating a subject suffering from a condition which may be alleviated by administration of a beneficial agent comprising administering to the subject a therapeutically effective amount of a formulation comprising at least one beneficial agent and a non-aqueous, single-phase biocompatible vehicle. The vehicle comprises the solvent lauryl lactate, a surfactant and a polymer, and the solvent, surfactant, and polymer are selected

and formulated such that the vehicle exhibits a viscosity capable of suspending the at least one beneficial agent.

In contrast to the claims of the present application, the Sherman reference discloses pharmaceutical compositions which enable improved absorption of a hydrophobic drug while, at the same time, enabling the drug to be contained in the composition at relatively high concentration. *See, Sherman Reference* at col. 3, lines 19–23. The pharmaceutical compositions are provided in the form of emulsions (or emulsion preconcentrates) in a solvent system comprising at least a solvent and a surfactant. The solvent comprises at least one alcohol having a boiling point above 100°C and a solubility in water below 10g per 100g at 20°C. *See id.* at col. 3, lines 34–54; Abstract. The Sherman reference, however, does not disclose a solvent system wherein the solvent is *lauryl lactate* as recited in amended independent claims 17, 18 and 36 of the present application. Accordingly, the Sherman reference does not describe each and every element as set forth in claims 17, 18 and 36 and, thus, these claims are not anticipated by the Sherman reference.

Further, it is respectfully submitted that Sherman reference does not disclose a non-aqueous, single-phase biocompatible vehicle *which exhibits a viscosity capable of suspending at least one beneficial agent*, as recited in independent claims 17, 18 and 36. It appears to be the Examiner's assertion that the teachings of the Sherman reference inherently anticipate the claims pending in the present application as it is stated in the outstanding Office Action that the recited viscosity is an inherent property "of the broad composition where the composition comprises a surfactant, solvent and polymer and active agent." *Office Action*, at page 4, ¶ 2. As the Examiner has not met the burden of establishing inherency, Applicants respectfully traverse this assertion.

In order to establish inherency, it is incumbent upon the Examiner to provide evidence that "must make clear that the missing descriptive matter is necessarily present in the thing described in the reference, and that it would be so recognized by persons of ordinary skill in the art." *In re Robertson*, 169 F.3d 743, 745, 49 UPSQ2d 1949, 1950-51 (Fed. Cir. 1999); MPEP §2112. That is, "[i]n relying on the theory of inherency, the examiner must provide a basis in fact and/or technical reasoning to reasonably support the determination that the allegedly inherent characteristic necessarily flows from the teachings of the applied prior art." *Ex parte Levy*, 17

USPQ2d 1461, 1464 (Bd. Pat. App. & Inter. 1990) (emphasis in original); MPEP § 2112. Significantly, inherency "may not be established by possibilities or probabilities. The mere fact that a certain thing may result from a given set of circumstances is not sufficient." *In re Robertson*, 169 F.3d 743, 745, 49 UPSQ2d 1949, 1950-51 (Fed. Cir. 1999); MPEP §2112. *See also, Continental Can Co. v. Monsanto Co.*, 948 F.2d 1264, 1269 (Fed. Cir. 1991). Therefore, in order for the Examiner to properly establish that the Sherman reference anticipates the rejected claims, the Examiner bears the burden of providing a basis in fact and/or technical reasoning that establishes that each time formulations as taught in Sherman are prepared, a vehicle or formulation as recited in the rejected claims necessarily results. Applicants respectfully submit that such a burden has not been met.

It is respectfully submitted that there is no basis in fact and/or technical reasoning that has been provided in the outstanding Office Action that reasonably supports a determination that the subject matter recited in the rejected claims necessarily flows from the teachings of the Sherman reference. In particular, the outstanding Office Action includes no evidence to support the assertion that every composition or formulation that comprises a solvent, a surfactant and a polymer would necessarily result in a non-aqueous, single-phase biocompatible vehicle or formulation *that exhibits a viscosity capable of suspending at least one beneficial agent.* Applicants respectfully submit that every composition or formulation that includes a combination of a solvent, a surfactant, and a polymer will *not* necessarily provide a vehicle or formulation that is non-aqueous, single-phase, biocompatible, and exhibits a viscosity capable of suspending at least one beneficial agent. Therefore, if it is to be asserted that the Sherman reference discloses a vehicle or formulation as recited in the rejected claims, the burden of establishing a reasonable basis for a finding of inherency remains with the Examiner.

As the Sherman reference fails to describe each and every element as set forth in amended independent claims 17, 18 and 36, Applicants respectfully submit that these claims are not anticipated by the Sherman reference. As each of claims 19 through 27, 29 through 31, 37, 52 and 53 depends, either directly or indirectly, from one of claims 17 and 36, Applicants respectfully submit that these claims are also not anticipated by the Sherman reference for at least the above-cited reasons. Accordingly, Applicants respectfully request withdrawal of the 35 U.S.C. § 102(b) rejection of claims 17, through 27, 29 through 31, 36, 37, 52 and 53 based upon

the Sherman reference. Each of these claims is believed to be in condition for allowance and such favorable action is respectfully requested.

**35 U.S.C. § 103(a) Rejection**

A. Applicable Authority

The basic requirements of a *prima facie* case of obviousness are summarized in MPEP §2143 through §2143.03. In order "[t]o establish a *prima facie* case of obviousness, three basic criteria must be met. First, there must be some suggestion or motivation, either in the references themselves or in the knowledge generally available to one of ordinary skill in the art, to modify the reference or combine the reference teachings. Second, there must be a reasonable expectation of success [in combining the references]. Finally, the prior art reference (or references when combined) must teach or suggest all the claim limitations. The teaching or suggestion to make the claimed combination and the expectation of success must both be found in the prior art, not in applicant's disclosure. *In re Vaeck*, 947 F.2d 488, 20 USPQ2d 1438 (Fed. Cir. 1991)." Further, in establishing a *prima facie* case of obviousness, the initial burden is placed on the Examiner. "To support the conclusion that the claimed invention is directed to obvious subject matter, either the references must expressly or impliedly suggest the claimed invention or the examiner must present a convincing line of reasoning as to why the artisan would have found the claimed invention to have been obvious in light of the teachings of the references." *Ex parte Clapp*, 227 USPQ 972, 973 (Bd. Pat. App. & Inter. 1985). *See also* MPEP §706.02(j) and §2142.

B. Obviousness Rejection of Claims 38, 40 and 49 Based on the Sherman Reference

Claims 38, 40, and 49 stand rejected under 35 U.S.C. §103(a) as being unpatentable over the Sherman reference (*Office Action*, at page 4). As the Sherman reference neither teaches nor suggests all of the limitations of claims 38, 40, and 49, Applicants respectfully traverse this rejection, as hereinafter set forth.

Each of claims 38 and 40 depend from amended independent claim 36 and claim 49 depends from amended independent claim 17. Each of claims 36 and 17 was discussed hereinabove, as was the Sherman reference. It is respectfully submitted that the Sherman

reference neither teaches nor suggests all of the limitations of independent claims 17 and 36 and, accordingly, a *prima facie* case of obviousness of claims 38, 40 and 49 cannot be established based upon the Sherman reference.

In particular, it is respectfully submitted that the Sherman reference fails to teach or suggest a formulation or vehicle comprising a surfactant, a polymer and the solvent lauryl lactate as recited in each of dependent claims 38, 40 and 49. Rather, as previously discussed, the Sherman reference teaches a solvent comprised of at least one alcohol having a boiling point above 100°C and a solubility in water below 10g per 100g at 20°C. *See, Sherman Reference* at col. 3, lines 34–54; Abstract. Further, it is respectfully submitted that the Sherman reference fails to teach or suggest a non-aqueous, single-phase biocompatible vehicle which exhibits a viscosity capable of suspending at least one beneficial agent as recited in the rejected claims. While the Examiner has asserted that the formulations of the Sherman reference inherently comprise these characteristics, it is respectfully submitted that the burden for establishing inherency has not been met. *See supra.*

As a *prima facie* case of obviousness of claims 38, 40 and 49 cannot be established based upon the Sherman reference, Applicants respectfully request withdrawal of the 35 U.S.C. §103(a) rejection of these claims. Each of claims 38, 40 and 49 is believed to be in condition for allowance and such favorable action is respectfully requested.

**Objections to Claims 33-35, 39 and 41/Allowable Subject Matter**

Claims 33 through 35, 39 and 41 stand objected to as being dependent upon rejected base claims, but are indicated to contain allowable subject matter and would be allowable if placed in appropriate independent form. Claim 33 has been amended herein and now appears in independent form. As such, independent claim 33, and corresponding dependent claims 34 and 35, are believed to be in condition for allowance and such favorable action is respectfully requested.

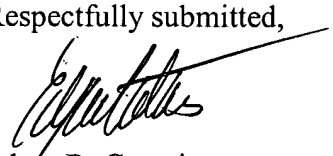
With respect to claims 39 and 41, Applicants note the indication of allowable subject matter with appreciation. However, as it is believed that in view of the amendments and comments provided herein, independent claim 36, from which each of claims 39 and 41 depend,

either directly or indirectly, is in condition for allowance, the dependency of claims 39 and 41 remains.

## CONCLUSION

Each of claims 17 through 27, 29 through 31, 33 through 41, and 49 through 53 is believed to be in condition for allowance, and an early notice thereof is respectfully solicited. If it is determined that additional issues remain which might be resolved by a telephone conference, the Examiner is respectfully invited to contact Applicants' undersigned attorney.

Respectfully submitted,



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Enclosures: Appendices A and B

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# APPENDIX A

**(CLEAN VERSION OF SUBSTITUTE SPECIFICATION EXCLUDING CLAIMS)**

**(Serial No. 09/627,531)**


PATENT
Attorney Docket 6169.1US

NOTICE OF EXPRESS MAILING

Express Mail Mailing Label Number: _____

Date of Deposit with USPS: _____

Person making Deposit: _____

APPLICATION FOR LETTERS PATENT

for

# STABLE NON-AQUEOUS SINGLE PHASE VISCOUS VEHICLES AND FORMULATIONS UTILIZING SUCH VEHICLES

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TITLE OF THE INVENTION

# STABLE NON-AQUEOUS SINGLE PHASE VISCOUS VEHICLES AND FORMULATIONS UTILIZING SUCH VEHICLES

## CROSS-REFERENCE TO RELATED APPLICATIONS

**[0001]** This application is a continuation-in-part application of U.S. application Serial No. 09/497,422, filed February 3, 2000.

**[0002]** Field of the Invention: This invention relates to stable nonaqueous single-phase biocompatible viscous vehicles capable of suspending beneficial agents and uniformly dispensing these agents at low flow rates and, more particularly, to stable uniformly mixed formulations of beneficial agents in stable nonaqueous single-phase biocompatible viscous vehicles.

## REFERENCES

**[0003]** The following references are referred to by numbers in brackets ([]) at the relevant portion of the specification.


1. Wang et al., *J. Parenteral Sci. Tech,* **42:** S4-S26 (1988).
2. Desai et al., *J. Am. Chem. Soc.,* **116**: 9420-9422 (1994).
3. Chang et al., *Pharm. Tech.,* 80-84 (Jan. 1996).
4. Manning et al., *Pharm. Res.,* **6**: 903-918 (1989).
5. Hageman *Drug Dev. Ind. Pharm,* **14**: 2047-2070 (1988).
6. Bell et al., *Biopolymers,* **35**: 201-209 (1995).
7. Zhang et al., *Pharm. Res.* **12**: 1447-1452 (1995).
8. PCT published application 98/00158
9. PCT published application 98/16250
10. Knepp et al., *Pharm. Res.* 15 (7) 1090-1095 (1998).
11. PCT published application 98/00157
12. PCT published application 98/00152

13. U.S. Patent 5,540,912
17. Yu et al., J. Pharm. Sci., 85: 396-401 (1996).
18. Mitchell, U.S. Patent No. 5,411,951 (1995).
19. Brooks et al., U.S. Patent No. 5,352,662 (1994)
20. Geller, L., U.S. Patent No. 3,869,549 (1975).
21. Larsen et al., PCT Publication No. WO95/34285 (1995).
22. Knepp et al., *J. Pharm. Sci. Tech,* **50**: 163-171 (1996).
23. U.S. Patent 5,614,221
24. U.S. Patent 4,594,108
25. U.S. Patent 5,300,302
26. U.S. Patent 4,588,614
27. U.S. Patent 4,310,516
28. U.S. Patent 5,635,213
29. EP 379,147

## BACKGROUND OF THE INVENTION

**[0004]** Peptides, polypeptides, proteins and other proteinaceous substances (e.g., viruses, antibodies) collectively referred to herein as "proteins", have great utility as pharmaceuticals in the prevention, treatment and diagnosis of disease. Proteins are naturally active in aqueous environments, thus, the preferred formulations of proteins have been in aqueous solutions. However, proteins are only marginally stable in aqueous solutions. Thus, protein pharmaceuticals often have short shelf-lives under ambient conditions or require refrigeration. Further, many proteins have only limited solubility in aqueous solutions. Even when they are soluble at high concentrations, they are prone to aggregation and precipitation.

**[0005]** Because proteins can easily degrade, the standard method for delivering such compounds has been daily injections. Proteins can degrade via a number of mechanisms, including deamidations of asparagine and glutamine; oxidation of methionine and, to a lesser degree, tryptophan, tyrosine and histidine; hydrolysis of peptide bonds; disulfide interchange; and racemization of chiral amino acid residues [1-7]. Water is a reactant in nearly all of these degradation pathways. Further, water acts as a plasticizer, which facilitates unfolding and irreversible aggregation of proteins. Since water is a participant in almost all protein degradation

pathways, reduction of aqueous protein solution to a dry powder provides an alternative formulation methodology to enhance the stability of protein pharmaceuticals.

**[0006]** One approach to stabilizing proteins is to dry them using various techniques, including freeze-drying, spray-drying, lyophilization, and desiccation. Dried proteins are stored as dry powders until their use is required.

**[0007]** A serious drawback to drying of proteins is that often one would like to use proteins in some sort of flowable form. Parenteral injection and the use of drug delivery devices for sustained delivery of drugs are two examples of the applications where one would like to use proteins in a flowable form. For injection, dried proteins must be reconstituted, adding additional steps which are time-consuming and where contamination may occur, and exposing the protein to potentially destabilizing conditions [7]. For drug delivery devices, the protein formulations must be stable for extended periods of time at body temperature and maintain their flowability for the expected life of the device.

**[0008]** Solution formulations of proteins/peptides in nonaqueous polar aprotic solvents such as DMSO and DMF have been shown to be stable at elevated temperatures for long periods of time [8]. However, such solvent- based formulations will not be usable for all proteins since many proteins have low solubility in these solvents. The lower the solubility of the protein in the formulation, the more solvent would have to be used for delivery of a specific amount of protein. Low concentration solutions may be useful for injections, but may not be useful for long-term delivery at low flow rates.

**[0009]** Proteins have been formulated for delivery using perfluorodecalin [9, 10], methoxyflurane [9], high concentrations in water [11], polyethylene glycol [12], PLGA [13, 14], ethylenevinylacetate/polyvinylpyrridone mixtures [15], and PEG400/povidone [16]. However, these formulations were not shown to retain a uniform suspension of protein in viscous vehicles over long periods of time.

**[0010]** Many biologically active compounds degrade over time in aqueous solution. Carriers in which proteins do not dissolve but rather are suspended can often offer improved chemical stability. Furthermore, it can be beneficial to suspend the beneficial agent in a carrier when the agent exhibits low solubility in the desired vehicle. However, suspensions can have poor physical stability due to settling and agglomeration of the suspended beneficial agent. The

problems with nonaqueous carriers tend to be exacerbated as the concentration of the active compound is increased.

**[0011]** Dispersing powdered proteins or peptides in lipid vehicles to yield parenteral sustained release formulations has been investigated [17-21]. The vehicles used were either various vegetable (sesame, soy, peanut, etc.) or synthetic oils (e.g., Miglyol) gelled with aluminum fatty acid esters such as aluminum stearates (mono-, di- or tri-), or with a polyglycerol ester. Although theoretically these vehicles might preclude solution denaturation and protect the drug from aqueous chemical degradation, the vehicles themselves are unstable at higher temperatures. The storage of liquid vegetable oils at body temperatures results in the formation of reactive species such as free fatty acids and peroxides (a process which is accelerated by the presence of traces of various metal ions such as copper or iron which can leach from some implantable devices). These peroxides not only adversely affect protein stability [22] but would be toxic when delivered directly to, for example, the central nervous system of a human or animal.

**[0012]** The sustained delivery of drugs has many advantages. Use of implantable devices assures patient compliance, since the delivery device is tamperproof. With one insertion of a device, rather than daily injections, there is reduced site irritation, fewer occupational hazards for practitioners, improved cost effectiveness through decreased costs of equipment for repeated injections, reduced hazards of waste disposal, and enhanced efficacy through controlled release as compared with depot injection. The use of implantable devices for sustained delivery of a wide variety of drugs or other beneficial agents is well known in the art. Typical devices are described, for example, in U.S. Patents Nos: 5,034,229; 5,057,318; 5,110,596; and 5,782,396. The disclosure of each of these patents is incorporated herein by reference.

**[0013]** For drug delivering implants, dosing durations of up to one year are not unusual. Beneficial agents which have low therapeutic delivery rates are prime candidates for use in implants. When the device is implanted or stored, settling of the beneficial agent in a liquid formulation can occur. This heterogeneity can adversely affect the concentration of the beneficial agent dispensed. Compounding this problem is the size of the implanted beneficial agent reservoir. Implant reservoirs are generally on the order of 25-250 $\mu$l, but can be up to 25 ml.

**[0014]** Viscous formulations have been prepared using two separate components to be mixed with a drug at use [23], thickening agents added to aqueous compositions [24], gelling agents added to aqueous drug solutions [25], porous textile sheet material [26], thickening agents with oleaginous material [27], viscous aqueous carriers for limited solubility drugs [28], and extrudable elastic gels [29]. However, these formulations are mixed at use, contain aqueous components, use sheet matrices, or are delivered topically, orally, or intraduodenally.

**[0015]** Stability of formulations can be enhanced by freeze-drying, lyophilizing or spray-drying the active ingredient. The process of drying the active ingredient includes further advantages such as the use of compounds that are relatively unstable in aqueous solution that can be processed and filled into dosage containers, dried without elevated temperatures, and then stored in the dry state in which there are relatively few stability problems.

**[0016]** Pharmaceutical formulations, particularly parenteral products, should be sterilized after being sealed in the final container and within as short a time as possible after the filling and sealing have been completed. (See, for example, Remington, Pharmaceutical Sciences, 15$^{th}$ ed. (1975)). Examples of sterilization techniques include thermal or dry-heat, aseptic, and ionized radiation. Combinations of these sterilization procedures may also be used to produce a sterile product.

**[0017]** There is a need to be able to deliver protein compositions to the body which are stable at body temperatures over extended periods of time to enable long-term delivery of the protein. There is a need to be able to deliver concentrations of proteins that are efficacious. There is a need for a novel nonaqueous formulation capable of homogeneously suspending proteins and dispensing such agents at body temperatures and low flow rates over extended periods of time.

## BRIEF SUMMARY OF THE INVENTION

**[0018]** The present invention provides stable single-phase nonaqueous biocompatible viscous vehicles capable of forming uniform suspensions with proteins. The components of the viscous vehicle comprise at least two of a polymer, a surfactant, and a solvent. The ratios of the components will vary depending on the molecular weight of the components and the desired viscosity of the final vehicle. Presently preferred component ratios are: polymer, about 5% to about 60%; solvent, about 5% to about 60%; and surfactant, about 5% to about 40%.

**[0019]** The present invention also provides stable formulations in which beneficial agents are uniformly suspended in stable single-phase nonaqueous biocompatible viscous vehicles. In particular, the beneficial agents are formulated in the viscous vehicles at concentrations of at least about 0.1%, depending upon the potency of the beneficial agent. These stable formulations may be stored at the temperature appropriate for the beneficial agent, ranging from cold to body temperature (about 37°C) for long periods of time (1 month to 1 year or more). In a preferred embodiment, the formulation comprises about 0.1 to 50% (w/w) of beneficial agent, depending on the potency of the beneficial agent, the duration of treatment, and the rate of release for the drug delivery system.

**[0020]** These formulations are especially useful in implantable delivery devices for long-term delivery (e.g., 1 to 12 months or longer) of beneficial agent at body temperature, preferably about 37°C. Thus, the present invention also provides for the delivery of such proteins to the body over an extended period of time to enable long-term delivery of the protein at low flow rates of about 0.3 to 100 $\mu$l/day, preferably about 0.3 to 4 $\mu$l/day for about a six-month delivery period and preferably 5 to 8 $\mu$l/day for about a three-month delivery period.

**[0021]** In another aspect, the invention provides methods for preparing stable nonaqueous biocompatible formulations of a beneficial agent in a single-phase viscous vehicle. Preferred formulations comprise about 0.1 to 50% (w/w) beneficial agent depending on the potency of the beneficial agent, the duration of treatment, and the rate of release from the delivery system.

**[0022]** In yet a further aspect, the invention provides methods for treating a subject suffering from a condition which may be alleviated by administration of a beneficial agent, these methods comprising administering to the subject an effective amount of a stable nonaqueous formulation comprising at least one beneficial agent uniformly suspended in a single-phase viscous vehicle.

**[0023]** A further aspect of the invention is that nonaqueous single-phase viscous vehicles containing beneficial agents are chemically and physically stable over a broad temperature range for long periods of time. The beneficial agents in the viscous vehicles are also chemically and physically stable over a broad temperature range for long periods of time. Thus, these formulations are advantageous in that they may be shipped and stored at temperatures below, at, or above room temperature for long periods of time. They are also suitable for use in

implantable delivery devices in which the formulation must be stable at body temperature for extended periods of time.

**[0024]** The formulations of the present invention also remain stable when delivered from implantable drug delivery systems. The beneficial agents have been shown to exhibit zero order release rates when delivered from implantable drug delivery systems at very low flow rates over extended periods of time.

## BRIEF DESCRIPTION OF THE SEVERAL VIEWS OF THE DRAWINGS

**[0025]** Figure 1 shows the stability of human growth hormone (hGH) formulations of the present invention as determined at 37° C by reverse phase HPLC.

**[0026]** Figure 2 shows the stability of hGH formulations of the present invention as determined at 37° C by size exclusion chromatography.

**[0027]** Figure 3 shows the average release rate (μl/day) of 10% (w/w) spray-dried lysozyme in formulations of the present invention.

**[0028]** Figure 4 shows the average release rate (μl/day) of 10% (w/w) spray-dried hGH in a glycerol monolaurate/lauryl lactate/polyvinylpyrrolidone vehicle.

**[0029]** Figure 5 shows the average release rate (μg/day) of 10% lysozyme in a lauryl alcohol/polyvinylpyrrolidone vehicle.

**[0030]** Figure 6 shows the average release rate (μg/day) of 25% lysozyme in a glycerol monolaurate/lauryl lactate/polyvinylpyrrolidone vehicle.

**[0031]** Figure 7 shows the average release rate (μg/day) of 33% lysozyme in a glycerol monolaurate/lauryl lactate/polyvinylpyrrolidone vehicle.

**[0032]** Figure 8 shows the average release rate μg/day) of 45% lysozyme in a glycerol monolaurate/lauryl lactate/polyvinylpyrrolidone vehicle.

## DETAILED DESCRIPTION OF THE INVENTION

**[0033]** The present invention is drawn to the unexpected discovery that uniformly suspending beneficial agents in nonaqueous single-phase biocompatible viscous vehicles results in stable formulations which can be delivered at body temperature over an extended period of time at low flow rates. Previously known formulations of beneficial agents which are buffered aqueous or nonaqueous solutions which may or may not contain excipients are not formulations

which can be uniformly dispensed at body temperatures at low flow rates over an extended period of time without exhibiting unacceptable amounts of aggregation or degradation of the formulation. The presently claimed formulations stabilize beneficial agents and can be stored at the temperature appropriate for the beneficial agent. The temperatures can range from cold (not exceeding 8°C) to body temperature (about 37°C) for long periods of time. These formulations are especially useful in implantable delivery devices for long-term delivery (e.g., 1 to 12 months or longer) of a drug at low flow rates and at body temperature, preferably about 37°C.

**[0034]** Standard beneficial agent formulations consist of dilute aqueous or nonaqueous solutions or suspensions. Drug stability is usually achieved by varying one or more of the following: pH, buffer type, ionic strength, excipients (EDTA, ascorbic acid, etc.) For these formulations, degradation pathways requiring water (hydrolysis, deamidation, racemization) cannot be fully stabilized. In the present invention, beneficial agents formulated in nonaqueous biocompatible single-phase viscous vehicles containing, for example, polyvinylpyrrolidone, vinyl acetate, and/or polyoxyethylenepolyoxypropylene block copolymers were shown to be chemically and physically stable. The viscosity of the formulation will depend upon a number of criteria, including the beneficial agent potency and concentration, and the process by which the formulation is prepared. The viscosity of the formulation can be chosen so that the desired amount of beneficial agent is delivered over the desired period of time.

**[0035]** The invention also consists of nonaqueous single-phase biocompatible viscous vehicles capable of uniformly suspending beneficial agents and formulations containing at least one beneficial agent uniformly suspended in the viscous vehicle. The invention also consists of formulations containing at least one beneficial agent uniformly suspended in a nonaqueous single-phase biocompatible viscous vehicle, which formulations are stable for an extended period of time at body temperature and capable of delivering the beneficial agents uniformly at low flow rates. The discovery consists of the realization that stable nonaqueous viscous vehicles improve the stability of beneficial agents in a wide range of formulation conditions including concentration, elevated temperatures and duration of stable formulation, thus making possible the delivery of beneficial agents in long-term implantable devices that would not otherwise be feasible.

DEFINITIONS

**[0036]** As used herein, the following terms have the following meanings:

The term "chemical stability" means that an acceptable percentage of degradation products produced by chemical pathways such as oxidation, deamidation, or hydrolysis is formed. In particular, a formulation is considered chemically stable if no more than about 35% breakdown products are formed after 2 months at 37°C.

**[0037]** The term "physical stability" means that an acceptable percentage of aggregates (e.g., dimers, trimers and larger forms) are formed by the beneficial agent. For the formulation (viscous vehicle and beneficial agent), this term means that the formulation retains stability, flowability, and the ability to uniformly dispense the beneficial agent. In particular, a formulation is considered physically stable if no more than about 15% aggregates are formed after two months at 37°C.

**[0038]** The term "stable formulation" means that at least about 65% chemically and physically stable beneficial agent remains after two months at 37°C (or equivalent conditions at an elevated temperature). Particularly preferred formulations are those which retain at least about 80% chemically and physically stable beneficial agent under these conditions. Especially preferred stable formulations are those which do not exhibit degradation after sterilizing irradiation (e.g., gamma, beta or electron beam).

**[0039]** The term "beneficial agent" means peptides, proteins, nucleotides, hormones, viruses, antibodies, etc. that comprise polymers of amino acid or nucleic acid residues. These beneficial agents are generally degradable in water and generally stable as a dry powder at elevated temperatures. Synthetically produced, naturally derived or recombinantly produced moieties are included in this term. The term also includes lipoproteins and post-translationally modified forms, e.g., glycosylated proteins. Analogs, derivatives, agonists, antagonists and pharmaceutically acceptable salts of any of these are included in this term. The term also includes proteins and/or protein substances which have D-amino acids, modified, derivatized or non-naturally occurring amino acids in the D- or L- configuration and/or peptomimetic units as part of their structure. The term "protein" will be used in the present invention. The term also means that the beneficial agent is present in the solid state, e.g., powder or crystalline.

**[0040]** The term "excipient" means a more or less inert substance in a formulation that is added as a diluent or vehicle or to give form or consistency. Excipients are distinguished from

solvents such as ETOH, which are used to dissolve drugs in formulations. Excipients include non-ionic surfactants such as polysorbates, which are used to solubilize drugs in formulations; preservatives such as benzyl alcohols or methyl or propyl parabens, which are used to prevent or inhibit microbial growth; chelating agents; flavoring agents; and other pharmaceutically acceptable formulation aides.

**[0041]** The term "viscous vehicle" means a vehicle with a viscosity in the range of about 1,000 to 10,000,000 poise. The term includes Newtonian and non-Newtonian materials. Preferred are vehicles with a viscosity of about 10,000 to 250,000 poise. The formulations of this invention can uniformly expel beneficial agents suspended in the viscous vehicle from implantable drug delivery devices. The formulations exhibit a shear rate at the exit of the devices of 1 to 1 x $10^{-7}$ reciprocal second, preferably an exit shear rate of 1 x $10^{-2}$ to 1 x $10^{-5}$ reciprocal second.

**[0042]** The term "single-phase" means a solid, semisolid, or liquid homogeneous system that is both physically and chemically uniform throughout as determined by differential scanning calorimetry (DSC). The DSC scan should show one peak indicative of a single-phase.

**[0043]** The term "biocompatible" means a property or characteristic of a viscous vehicle to disintegrate or break down, over a prolonged period of time, in response to the biological environment in the patient, by one or more physical or chemical degradative processes, for example, by enzymatic action, oxidation or reduction, hydrolysis (proteolysis), displacement, e.g., ion exchange, or dissolution by solubilization, emulsion or micelle formation, and which material is then absorbed by the body and surrounding tissue, or otherwise dissipated thereby.

**[0044]** The term "polymer" includes polyesters such as PLA (polylactic acid) having an inherent viscosity in the range of about 0.5 to 2.0 i.v. and PLGA (polylacticpolyglycolic acid) having an inherent viscosity in the range of about 0.5 to 2.0 i.v., pyrrolidones such as polyvinylpyrrolidone (having a molecular weight range of about 2,000 to 1,000,000), esters or ethers of unsaturated alcohols such as vinyl acetate, and polyoxyethylenepolyoxypropylene block copolymers (exhibiting a high viscosity at 37°C) such as Pluronic 105. A currently preferred polymer is polyvinylpyrrolidone.

**[0045]** The term "solvent" includes carboxylic acid esters such as lauryl lactate, polyhydric alcohols such as glycerin, polymers of polyhydric alcohols such as polyethylene

glycol (having a molecular weight of about 200 to 600), fatty acids such as oleic acid and octanoic acid, oils such as castor oil, propylene carbonate, lauryl alcohol, or esters of polyhydric alcohols such as triacetin acetate. Currently preferred is lauryl lactate.

**[0046]** The term "surfactant" includes esters of polyhydric alcohols such as glycerol monolaurate, ethoxylated castor oil, polysorbates (for example, Polysorbate 80), esters or ethers of saturated alcohols such as myristyl lactate (Ceraphyl 50), and polyoxyethylenepolyoxypropylene block copolymers such as Pluronic (for example, F68). Currently preferred are gylcerol monolaurate and polysorbates.

**[0047]** The term "antioxidant" means a pharmaceutically acceptable aid for stabilization of the beneficial agent against degradation such as oxidation. Antioxidants include, but are not limited to, tocopherol (vitamin E), ascorbic acid, ascorbyl palmitate, butylated hydroxyanisole, butylated hydroxytoluene, and propyl gallate. A preferred antioxidant depends on solubility and the efficiency of the antioxidant for protecting against degradation or chemical change of the beneficial agent in the preferred vehicle. Currently preferred is ascorbyl palmitate.

PREPARATION OF FORMULATIONS

**[0048]** The present invention is drawn to stable nonaqueous single-phase biocompatible viscous vehicles capable of suspending beneficial agents and uniformly dispensing the beneficial agents at body temperature at low flow rates over an extended period of time. The present invention is also directed to formulations containing beneficial agents uniformly suspended in the single-phase biocompatible viscous vehicles which are stable for prolonged periods of time at bodytemperature.

**[0049]** Examples of beneficial agents that may be formulated using the present invention include those peptides or proteins that have biological activity or that may be used to treat a disease or other pathological condition. They include, but are not limited to, adrenocorticotropic hormone, angiotensin I and II, atrial natriuretic peptide, bombesin, bradykinin, calcitonin, cerebellin, dynorphin N, alpha and beta endorphin, endothelin, enkephalin, epidermal growth factor, fertirelin, follicular gonadotropin releasing peptide, galanin, glucagon, GLP-1, gonadorelin, gonadotropin, goserelin, growth hormone releasing peptide, histrelin, human growth hormone, insulin, interferons, leuprolide, LHRH, motilin, nafarerlin, neurotensin, oxytocin, relaxin, somatostatin, substance P, tumor necrosis factor,

triptorelin, vasopressin, growth hormone, nerve growth factor, blood clotting factors, ribozymes, and antisense oligonucleotides. Analogs, derivatives, agonists and pharmaceutically acceptable salts of the above may also be used.

**[0050]** The beneficial agents useful in the formulations and methods of the present invention can be used in the form of a salt, preferably a pharmaceutically acceptable salt. Useful salts are known to those of skill in the art and include salts with inorganic acids, organic acids, inorganic bases, or organic bases.

**[0051]** Beneficial agents that are not readily soluble in nonaqueous solvents are preferred for use in the present invention. One of skill in the art can easily determine which compounds will be useful on the basis of their solubility. The amount of beneficial agent may vary depending on the potency of the compound, the condition to be treated, the solubility of the compound, the expected dose and the duration of administration. (See, for example, Gilman et al., The Pharmacological Basis of Therapeutics, $7^{th}$ ed. (1990) and Remington, Pharmacological Sciences, $18^{th}$ ed. (1990), the disclosures of which are incorporated herein by reference.)

**[0052]** It has been unexpectedly found that using a stable nonaqueous single-phase biocompatible viscous vehicle increases the stability of the beneficial agent. For example, as seen in Figures 1 and 2, human growth hormone (hGH) was found to be stable at 37°C over 12 weeks in formulations of polyvinylpyrrolidone/PEG; Pluronic; and glycerol monolaurate/lauryl lactate/polyvinylpyrrolidone. Figure 1 shows stability results using reverse phase HPLC. Figure 2 shows stability results using size exclusion chromatography.

**[0053]** Generally, stable nonaqueous single-phase biocompatible viscous vehicles may be prepared by combining the dry (low moisture content) ingredients in a dry box or under other dry conditions and blending them at elevated temperature, preferably about 40 to about 70°C, to allow them to liquify. The liquid vehicle is allowed to cool to room temperature. Differential scanning calorimetry was used to verify that the vehicle was single-phase. The final moisture content of the viscous vehicle was <2%.

**[0054]** Generally, the stable formulations of the present invention may be prepared by combining the vehicle and beneficial agent under dry conditions and blending them under vacuum at elevated temperature, preferably about 40 to about 70°C, to disperse the beneficial agent uniformly throughout the vehicle. The formulation is allowed to cool to room temperature.

**[0055]** It has been found that drying the beneficial agent prior to formulation enhances the stability of the formulation.

**[0056]** It has also been found that adding antioxidants, such as tocopherol, ascorbic acid, ascorbyl palmitate, butylated hydroxyanisole, butylated hydroxytoluene, and propyl gallate reduces the formation of degradation products (e.g., unstable chemical intermediates) during sterilization.

Methodology

**[0057]** We have found that stable nonaqueous beneficial agent formulations utilizing viscous vehicles may be prepared by combining the ingredients for the viscous vehicle under dry conditions and blending them at elevated temperature to allow them to liquify and form a single-phase. Once a single-phase viscous vehicle is formed, the vehicle is allowed to cool to room temperature. A beneficial agent is added with mixing at elevated temperature under vacuum to uniformly disperse it in the viscous vehicle.

**[0058]** We have tested these beneficial agent formulations, for example, formulations of hGH, for stability by subjecting them to accelerated aging tests. Results show that these formulations remained stable over extended periods of time.

**[0059]** We have tested beneficial agent formulations, for example, human growth hormone and lysozyme, for stability by suspending them in a variety of nonaqueous single-phase viscous vehicles prepared according to the present invention, then subjecting them to accelerated aging at elevated temperatures. The stability of the formulations was measured. Results of these studies demonstrate that these formulations were stable at conditions that approximate or exceed storage for one year at 37°C.

**[0060]** We have also tested beneficial agent formulations prepared as described herein for stability after 2.5 megarads gamma irradiation. Results show that these formulations remained chemically and physically stable after such irradiation.

METHODS

**[0061]** The following methods were used to perform the studies in the examples that follow.

1. Preparing Protein Powders

Human Growth Hormone (obtained, for example, from BresaGen Limited, Adelaide, Australia)

**[0062]** The active agent was reconstituted in deionized water. The solution containing the active agent was buffer exchanged using an Amicon Diaflo® Ultrafiltration membrane (molecular weight cut-off 10,000).

**[0063]** The diafiltrated active agent solution was spray dried using a Yamato mini-spray dryer. Powder was collected in a collection vessel through a cyclone trap. All handling of the spray-dried powder took place in a dry box evacuated with nitrogen. The generated powder was analyzed for particle size and distribution, moisture content, protein content and stability by size exclusion and reverse-phase chromatography.

**[0064]** It is known that the conformation of some proteins can be stabilized by the addition of a sugar (such as sucrose or mannitol) or a polyol (such as ethylene glycol, glycerol, glucose, and dextran.)

2. Preparation of Viscous Vehicles

**[0065]** We have found that stable single-phase biocompatible viscous vehicles may be prepared by combining the ingredients and blending them at elevated temperatures to allow them to liquify and form a single-phase. A differential scanning calorimetry scan showed one peak, indicative of a single-phase. The mixing was completed under vacuum to remove trapped air bubbles produced from the powders. The mixer was a dual helix blade mixer (D.I.T.) which runs at a speed around 40 rpm. Higher speeds can be used but are not required.

**[0066]** If a three-component viscous vehicle was prepared, the solvent portion of the vehicle was added to the heated bowl of the mixer first, followed by the surfactant. The polymer was added last, and the ingredients were mixed until a solution (single-phase) resulted. Vacuum was applied during mixing to remove air bubbles. The solution was dispensed from the bowl while at elevated temperature and allowed to cool to room temperature. On cooling, the vehicle

exhibited increased viscosity. Two- and single-component gels were made using the same process.

3. Preparation of Beneficial Agent Formulations

**[0067]** To prepare the formulation, the single-phase viscous vehicle was heated and then blended under vacuum with a weighed amount of beneficial agent. The beneficial agent and the single-phase viscous vehicle were blended in the same manner as the vehicle was prepared, using a dual helix blade mixer (or other similar mixer), at a speed between 40 and 120 rpm for approximately 15 minutes or until a uniform dispersion was attained. The resulting mixture was removed from the mixer, sealed in a dry container, and allowed to cool to room temperature.

4. Preparation of Reservoirs

**[0068]** The reservoirs of implantable drug delivery devices (as disclosed in U.S. Patent Application Serial No. 08/595,761, incorporated herein by reference) were filled with the appropriate hGH formulation. The formulation was filled into titanium reservoirs with a polymer plug blocking each end. The filled reservoir was then sealed in a polyfoil bag and placed in a stability testing oven.

**[0069]** It should be noted that the formulations in the reservoirs of these devices are completely isolated from the outside environment.

5. Reverse Phase-HPLC (RP-HPLC)

**[0070]** All stability samples of hGH were assayed for protein content and chemical stability by reverse phase chromatography (RP-HPLC). Analyses were performed on a Hewlett Packard HP-1090 system with a refrigerated autosampler (4°C). The chromatographic conditions used are listed below.

[0071] TABLE 1

RP-HPLC Chromatographic Conditions

| Description | Parameter | | |
|---|---|---|---|
| Column | J.T. Baker-C18, 4.6x250 mm | | |
| Flow Rate | 1.0 mL/min | | |
| Detection | 214 nm | | |
| Mobile Phase | A: 0.1% TFA in water | | |
| | B: 0.1% TFA in acetonitrile | | |
| Gradient | time | %A | %B |
| | 0 | 65 | 35 |
| | 5 | 50 | 50 |
| | 45 | 35 | 65 |
| | 50 | 30 | 70 |
| | 55 | 65 | 35 |

**[0072]** An hGH reference standard solution was prepared and its protein content calculated from the absorbance measurement at 280 nm. Three dilutions of this solution, representing 80%, 100%, and 120% of the expected concentration of hGH in the samples, were run in duplicate at the beginning and the end of each run and used to calculate the total protein content of the samples.

6. Size Exclusion Chromatography (SEC)

**[0073]** All stability samples of hGH were assayed for protein content and high-molecular-weight degradation products by size exclusion chromatography. Analyses were performed on a Hewlett Packard HP-1090 system with a refrigerated autosampler (4°C). The chromatographic conditions used are listed below

[0074] TABLE 2

SEC Chromatographic Conditions

| Description | Parameter |
|---|---|
| Column | TSK-2000SWXL |
| Flow Rate | 0.5 ml/min. |
| Detection | 214 nm |
| Mobile Phase | 25 mM sodium phosphate, 100 mM sodium chloride, pH 7.0 |

[0075] An hGH reference standard solution was prepared and its protein content calculated from the absorbance measurement at 280 nm. Three dilutions of this solution, representing 80%, 100%, and 120% of the expected concentration of hGH in the samples, were run in duplicate at the beginning and the end of each run and used to calculate the total protein content of the samples. The amount of high-molecular-weight degradation products was calculated by area normalization.

[0076] The following examples are offered to illustrate this invention and are not meant to be construed in any way as limiting the scope of this invention.

EXAMPLE 1

Preparation of Nonaqueous Single-Phase Viscous Vehicles

[0077] The nonaqueous single-phase viscous vehicles can be prepared as follows and shown in the table below.

[0078] A. Glycerol monolaurate (Danisco Ingredients, New Century,KS) (25 g) was dissolved in lauryl lactate (ISP Van Dyk Inc., Belleville, NJ) (35 g) at 65°C. Polyvinylpyrrolidone C30 (BASF, Mount Olive, NJ) (40 g) was added and the mixture blended at about 40 rpm in a dual helix blade mixer (D.I.T.) until a single-phase was achieved. Trapped air bubbles were removed by applying vacuum to the mixing chamber. The single-phase vehicle was dispensed from the mixer and allowed to cool to room temperature.

**[0079]** B. Glycerol monolaurate (Danisco Ingredients, New Century,KS) (25 g) was dissolved in lauryl lactate (ISP Van Dyk Inc., Belleville, NJ) (35 g) at 65°C. Polyvinylpyrrolidone C17 (BASF, Mount Olive, NJ) (40 g) was added and the mixture blended at about 40 rpm in a dual helix blade mixer (D.I.T.) until a single-phase was achieved. Trapped air bubbles were removed by applying vacuum to the mixing chamber. The single-phase vehicle was dispensed from the mixer and allowed to cool to room temperature.

**[0080]** C. Polyvinylpyrrolidone C30 (BASF, Mount Olive, NJ) (50 g) was dissolved in polyethylene glycol 400 (Union Carbide) (50 g) at approximately 65°C until a single-phase solution was formed. The single-phase vehicle was dispensed from the mixer and allowed to cool to room temperature.

**[0081]** D. Polyvinylpyrrolidone C17 (BASF, Mount Olive, NJ) (50 g) was dissolved in polyethylene glycol 400 (Union Carbide) (50g) at approximately 65°C until a single-phase solution was formed. The single-phase vehicle was dispensed from the mixer and allowed to cool to room temperature.

**[0082]** E. Polyvinylpyrrolidone C17 (BASF, Mount Olive, NJ) (50 g) was dissolved in castor oil (Spectrum, Gardena, CA) (50 g) at approximately 65°C until a single-phase solution was formed. The single-phase vehicle was dispensed from the mixer and allowed to cool to room temperature.

**[0083]** F. Polyvinylpyrrolidone C17 (BASF, Mount Olive, NJ) (50 g) was dissolved in octanoic acid (Spectrum, Gardena, CA) at approximately 65°C until a single-phase solution was formed. The single-phase vehicle was dispensed from the mixer and allowed to cool to room temperature.

**[0084]** G. Polyvinylpyrrolidone C17 (BASF, Mount Olive, NJ) (50 g) was dissolved in oleic acid (Spectrum, Gardena, CA) at approximately 65°C until a single-phase solution was formed. The single-phase vehicle was dispensed from the mixer and allowed to cool to room temperature.

**[0085]** H. Polyvinylpyrrolidone C17 (BASF, Mount Olive, NJ) (35%) was dissolved in glycerin (Baker, NJ) (65%) at approximately 65°C until a single-phase solution was formed. The single-phase vehicle was dispensed from the mixer and allowed to cool to room temperature.

**[0086]** I. Cremophor EL (ethoxylated castor oil) (BASF, Mount Olive, NJ) (5%) was dissolved in castor oil (Spectrum, Gardena, CA) (70%), and polyvinylpyrrolidone C17

(BASF, Mount Olive, NJ) (25%) was added and dissolved by mixing at approximately 40 rpm to form a single-phase vehicle. The single-phase vehicle was dispensed from the mixer and allowed to cool to room temperature.

**[0087]** J. Pluronic 105 (BASF, Mount Olive, NJ) was heated to approximately 65°C with mixing until melted. The single-phase vehicle was dispensed from the mixer and allowed to cool to room temperature.

**[0088]** K. Pluronic F68 (BASF, Sigma) 10% w/w and butylhydroxytoluene (Spectrum) 1% w/w were dissolved in 49% w/w propylene carbonate (Aldrich) by mixing under vacuum at 60°C until the materials dissolved. The vacuum was released, and the resulting liquid was added to 40% w/w polylactic acid (poly(D,L-lactide), Resomer R207, Boehringer Ingelheim). All components were mixed by hand at 60°C using a spatula until the polylactic acid was dissolved to form a single-phase vehicle. The single-phase vehicle was moved to a vacuum chamber to remove remaining air bubbles and allowed to cool to room temperature.

**[0089]** L. Myristyl lactate (Ceraphyl 50, ISP Van Dyk) 20% w/w was dissolved in 25 percent w/w lauryl alcohol (Sigma) under vacuum at 60°C. The vacuum was released, and the resulting liquid was added to a mixing bowl. Fifty-five percent w/w polyvinylpyrrolidone (BASF, 17pf) was added on top and the contents of the bowl were mixed at 40 rpm at 60°C under vacuum until all components were miscible and formed a single-phase vehicle. Vacuum was applied until air bubbles were removed from the single-phase vehicle.

**[0090]** Table 3

Component Ratios

| Polymer | Component Surfactant | Solvent | Ratio | Viscosity at Low Shear Rate (Poise) |
|---|---|---|---|---|
| PVP | GML | LL | 53:5:42 | 25,000 |
| PVP | GML | LL | 55:10:35 | 50,000 |
| PVP | GML | LL | 50:15:35 | 7,000 |
| PVP | ----- | LA | 60:40 | |
| PVP | Ceraphyl 50 | LA | 60:10:30 | |
| PVP | ----- | oleic acid | 50:50 | 30,000 |
| PVP | ----- | octanoic acid | 55:45 | 7,000 |
| PVP | polysorbate 80 | ----- | 50:50 | |

| | | | | |
|---|---|---|---|---|
| PVP | ----- | PEG 400 | 50:50 | |
| PVP | caster oil | ----- | 50:50 | |
| ----- | Pluronic 105 | ----- | 100 | 1,000,000 |
| PVP | ----- | glycerin | 50:50 | 5,000 |
| PLA | F68 | PC | 30:10:60* | |
| PVP (C17) | ML | LA | 50:25:25 | |
| PVP (C17) | polysorbate 80 | LL | 55:40:5 | |

**[0091]** Wherein:

GML = glycerol monolaurate

LL = lauryl lactate

PVP = polyvinylpyrrolidine C30

LA = lauryl alcohol

PEG = polyethyleneglycol 400

F68 = poly(propylene oxide)/poly(ethylene oxide) block copolymer (a member of the Pluronic family)

PC = propylene carbonate

PLA = polylactic acid

ML = myristyl lactate

* also contains 1% butylhydroxytoluene

## EXAMPLE 2

### Preparation of hGH

A. Preparation by Spray Drying

**[0092]** Lyophilized hGH (BresaGen Limited, Adelaide, Australia) was reconstituted in 150 ml of deionized water. This stock solution contained 1050 mg of hGH. Buffer exchange was accomplished using an Amicon Diaflo® Ultrafiltration membrane (molecular weight cut-off 10,000). The ultrafiltration cell was connected to an auxiliary reservoir containing 5mM phosphate buffer (pH 7). The cell's fluid volume, as well as the hGH concentration, remained constant as excipients were replaced by phosphate buffer.

**[0093]** The diafiltrated protein solution (protein concentration in the solution approximately 2%) was spray dried using a Yamato mini-spray dryer. Settings on the spray dryer were as follows: aspiration pressure constantly adjusted to 1.3 kgf/cm$^2$, inlet temperature 120°C, solution flow rate 2.5 (approximately 3 ml/min). Powder was collected in a collection vessel through a cyclone trap. All handling of the spray-dried powder took place in a dry box evacuated with nitrogen (% RH: 1-4%). The water content of the suspending vehicles is shown in the below table.

**[0094]** TABLE 4

WATER CONTENT OF SUSPENDING VEHICLES

| Vehicle | Water Content of Vehicle at T 0 % w/w | Water Content of Vehicle in 12 wks. at 37° C %w/w |
|---|---|---|
| Pluronic 105 | 0.25 | 0.4 |
| GML/LL/PVP | 1.5 | 1.3 |
| PVP/PEG | 2.0 | 2.0 |

**[0095]** Wherein:

GML = glycerol monolaurate

LL = lauryl lactate

PVP = polyvinylpyrrolidine C30

PEG = polyethyleneglycol 400

EXAMPLE 3

Preparation of hGH Formulation

**[0096]** A portion of the single-phase viscous vehicle was weighed (9 g) and heated to 60°C. hGH (BresaGen Limited, Adelaide, Australia) (1 g) was added to the vehicle and mixed for 15 minutes. The mixing was completed under vacuum to remove air bubbles added from the powder.

**[0097]** Approximately 10 mg of the spray-dried hGH powder were weighed out (content of hGH in the powder was recalculated based on the determined water and salt content) and mixed with 100 $\mu$l of the vehicle at 55-65°C (3 samples per each vehicle). Special care was taken while mixing powder in the suspending vehicle to achieve maximum particle uniform dispersion in the vehicle. All steps were done in a dry box.

**[0098]** The resulting suspension was dissolved with 10 ml of release rate buffer and analyzed by size exclusion and reverse-phase chromatography. Spray dried hGH powder was used as a control.

**[0099]** TABLE 5

STABILITY OF hGH SUSPENSIONS AT 37°C AS MEASURED BY SIZE EXCLUSION CHROMATOGRAPHY

| Time Weeks | Spray-dried Powder -80°C %LS | PVP/PEG 400 Suspension %LS | GML/LL/PVP Suspension %LS | Pluronic 105 Suspension %LS |
|---|---|---|---|---|
| 0 | 96±1 | 88±6 | 92±2 | 87±7 |
| 1 | 99±8 | 81±2 | 94±3 | 93±3 |
| 2 | 99±3 | 83±1 | 97±1 | 94±1 |
| 3 | 97±1 | 84±2 | 95±2 | 95±3 |
| 4 | 95±2 | 82±8 | 94±4 | 93±5 |
| 7 | 95±4 | 76±3 | 93±4 | 88±2 |
| 12 | 97±4 | 79±3 | 97±1 | 95±6 |

Each data point represents the mean ± relative standard deviation of three individual samples taken from three separate vials.

[00100] TABLE 6

STABILITY OF hGH SUSPENSIONS at 37°C AS MEASURED BY REVERSE PHASE CHROMATOGRAPHY

| Time Weeks | Spray-dried Powder -80°C %LS | PVP/PEG 400 Suspension %LS | GML/LL/PVP Suspension %LS | Pluronic 105 Suspension %LS |
|---|---|---|---|---|
| 0 | 104±1 | 99±3 | 99±2 | 89±7 |
| 1 | 104±8 | 78±2 | 98±3 | 96±6 |
| 2 | 104±4 | 73±3 | 95±1 | 96±1 |
| 3 | 104±2 | 78±4 | 97±3 | 97±4 |
| 4 | 100±2 | 74±10 | 93±4 | 96±4 |
| 7 | 108±5 | 72±4 | 96±2 | 94±2 |
| 9 | 102±3 | 66±3 | 92±3 | 93±2 |
| 12 | 101±2 | 66±1 | 89±2 | 92±5 |

Each data point represents the mean ± relative standard deviation of three individual samples taken from three separate vials.

## EXAMPLE 4

### Preparation of Reservoirs

### Release Rate Profiles

**[00101]** Titanium reservoir systems of implantable drug delivery devices (as disclosed in U.S. Patent Application Serial No. 08/595,761, incorporated herein by reference) were each assembled with an osmotic engine, piston, and rate-controlling membrane. The reservoirs were filled with the appropriate amount of viscous vehicle formulation and capped with a flow plug. The systems were placed in a water bath at 37° C and allowed to release formulation for an extended period of time. Released material was sampled twice per week. Assays for released material were completed using reverse phase HPLC. The resulting concentrations of beneficial agent for each system were converted to the released amount per day. The beneficial agent was

found to have a zero order release from the implantable drug delivery device, as shown in Figures 3 through 8.

## EXAMPLE 5

Stability of hGH in Nonaqueous Viscous Vehicle Formulations

**[00102]** Formulations of 10% w/w hGH in vehicle were prepared as described above and placed in vials. The formulations were subjected to accelerated aging by storing them at elevated temperatures and times in a controlled temperature oven shown in the table below.

**[00103]** TABLE 7

| Vehicle | Time(hrs) | Temperature | %LS by SEC | %LS by RP-HPLC |
|---|---|---|---|---|
| Pluronic 105 | 0 | 50°C | 98±3 | 101±3 |
| Pluronic 105 | 1 | 50°C | 98±3 | 101±4 |
| Pluronic 105 | 2 | 50°C | 100±1 | 102±3 |
| Pluronic 105 | 4 | 50°C | 101±3 | 105±3 |
| GML/LL/PVP | 0 | 65°C | 99±3 | 101±3 |
| GML/LL/PVP | 1 | 65°C | 93±6 | 97±6 |
| GML/LL/PVP | 2 | 65°C | 91±5 | 95±5 |
| GML/LL/PVP | 4 | 65°C | 95±3 | 98±3 |

Each data point represents the mean ± relative standard deviation of three individual samples taken from three separate vials.

**[00104]** Results, presented in the following table, demonstrate that these formulations were able to maintain the stability of the hGH in each case. In each case, at least 70% hGH was retained.

**[00105]** TABLE 8

RECOVERY OF hGH FROM NONAQUEOUS SUSPENSIONS

| Vehicle | %LS by RP-HPLC | %LS by Size-exclusion HPLC |
|---|---|---|
| PVP/PEG 400 | 99±3% | 88±6% |
| GML/LL/PVP | 99±2% | 92±2% |
| Pluronic 105 | 89±7% | 87±7% |

Each data point represents the mean ± relative standard deviation of three individual samples taken from three separate vials.

%LS or % label strength = (measured protein content + theoretical protein content) x 100%

EXAMPLE 6

A. Preparation by Spray Drying

**[00106]** GLP-1 (Polypeptide Laboratories, Wofenbuttel, Germany) was obtained as an acetate salt and was lyophilized. The lyophilized GLP-1 was dissolved in purified water at 19.9 mg/ml and spray dried using a Yamato mini-spray dryer. The spray drying parameters were: 120°C inlet temperature, 90°C outlet temperature, solution flow rate 3.3-5.3 ml/min. Powder was collected in a collection vessel through a cyclone trap. All handling of the spray-dried powder took place in a dry box evacuated with nitrogen (% RH: 1-4%).

B. Preparation of GLP-1 Formulation

**[00107]** A portion of the single-phase viscous vehicle was weighed and heated to 60°C. GLP-1 (Polypeptide Laboratories, Wolfenbuttel, Germany) was added 27% w/w to the vehicle and mixed for 15 minutes. The mixing was completed under vacuum to remove air bubbles.

**[00108]** The resulting suspension was dissolved in 10 ml of release rate buffer and analyzed by size exclusion and reverse-phase chromatography.

C. Analysis of GLP-1 Formulations

**[00109]** The reverse-phase HPLC method consisted of a C-8 5 $\mu$, 4.6 x 250 mm analytical column (Higgins Analytical, Mountain View, CA) with detection at 210 nm. A step gradient method from 25% B to 80% B at 1 ml/min. was as follows: 0-5 min. at 25% B, 5-30 min. at 25-50% B, 30-35 min. at 50-80% B. Mobile phase A was 0.1% TFA in water and mobile phase B was 0.1% TFA in acetonitrile. The formulations were found to be stable for 6 months.

**[00110]** The size exclusion chromatography method consisted of a Pharmacia FPLC HR 10/30 column at a flow rate of 0.5 ml/min. An isocratic method was employed, where the mobile phase was 100 mM ammonium phosphate, 200 mM sodium chloride, pH 2.0, and peptide was detected at 210 nm. The formulations were found to be stable for 6 months.

D. Preparation of Reservoirs

**[00111]** Titanium reservoir systems of implantable drug delivery devices (as disclosed in U.S. Patent Application Serial No. 08/595,761, incorporated herein by reference) were each assembled with an osmotic engine, piston, and rate-controlling membrane. The reservoirs were filled with the appropriate amount of viscous vehicle formulation and capped with a flow plug. The systems were placed in a water bath at 37° C and allowed to release formulation for an extended period of time. Released material was sampled twice per week. Assays for released material were completed using reverse phase HPLC. The resulting concentrations of beneficial agent for each system were converted to release amount per day. The beneficial agent was found to have a zero order release from the implantable drug delivery device.

**[00112]** Modification of the above-described modes of carrying out various embodiments of this invention will be apparent to those of skill in the art following the teachings of this invention as set forth herein. The examples described above are not limiting, but are merely exemplary of this invention, the scope of which is defined by the following claims.

## ABSTRACT OF THE DISCLOSURE

This invention relates to stable nonaqueous single-phase viscous vehicles and to formulations utilizing such vehicles. The formulations comprise at least one beneficial agent uniformly suspended in the vehicle. The formulation is capable of being stored at temperatures ranging from cold to body temperature for long periods of time. The formulations are capable of being uniformly delivered from drug delivery systems at an exit shear rate of between about 1 and 1 x $10^{-7}$ reciprocal second.


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# APPENDIX B

**(VERSION OF SUBSTITUTE SPECIFICATION EXCLUDING CLAIMS WITH MARKINGS TO SHOW CHANGES MADE)**

**(Serial No. 09/627,531)**

PATENT
Attorney Docket 6169.1US

NOTICE OF EXPRESS MAILING

Express Mail Mailing Label Number: _____

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Person making Deposit: _____

APPLICATION FOR LETTERS PATENT

for

# STABLE NON-AQUEOUS SINGLE PHASE VISCOUS VEHICLES AND FORMULATIONS UTILIZING SUCH VEHICLES

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TITLE OF THE INVENTION

# STABLE NON-AQUEOUS SINGLE PHASE VISCOUS VEHICLES AND FORMULATIONS UTILIZING SUCH VEHICLES

## CROSS-REFERENCE TO RELATED APPLICATIONS

**[0001]** This application is a continuation-in-part application of ~~US~~ U.S. application Serial No. ~~09/497,422~~ 09/497,422, filed February 3, 2000.

~~Field of the Invention~~

**[0002]** Field of the Invention: This invention relates to stable ~~non-aqueous~~nonaqueous ~~single phase~~single-phase biocompatible viscous vehicles capable of suspending beneficial agents and uniformly dispensing ~~said~~ these agents at low flow rates ~~and~~ and, more ~~particularly~~ particularly, to stable uniformly mixed formulations of beneficial agents in stable ~~non-aqueous~~nonaqueous ~~single phase~~single-phase biocompatible viscous vehicles.

~~References~~

## REFERENCES

**[0003]** The following references are referred to by numbers in brackets ([]) at the relevant portion of the specification.


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21. ~~Larsen,~~ Larsen et al., PCT Publication No. WO95/34285 (1995).

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23. U.S. Patent 5,614,221

24. U.S. Patent 4,594,108

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27. U.S. Patent 4,310,516

28. U.S. Patent 5,635,213

29. EP 379,147

~~Background of the Invention~~

## BACKGROUND OF THE INVENTION

**[0004]** Peptides, polypeptides, proteins and other proteinaceous substances (e.g., viruses, antibodies) collectively referred to herein as ~~proteins~~ "proteins", have great utility as pharmaceuticals in the prevention, treatment and diagnosis of disease. Proteins are naturally active in aqueous environments, ~~thus~~ thus, the preferred formulations of proteins have been in aqueous solutions. However, proteins are only marginally stable in aqueous solutions. Thus, protein pharmaceuticals often have short shelf-lives under ambient conditions or require refrigeration. Further, many proteins have only limited solubility in aqueous solutions. Even when they are soluble at high concentrations, they are prone to aggregation and precipitation.

**[0005]** Because proteins can easily degrade, the standard method for delivering such compounds has been daily injections. Proteins can degrade via a number of mechanisms, including deamidations of asparagine and glutamine; oxidation of methionine and, to a lesser degree, tryptophan, tyrosine and histidine; hydrolysis of peptide bonds; disulfide interchange; and racemization of chiral amino acid residues [1-7]. Water is a reactant in nearly all of these

degradation pathways. Further, water acts as a plasticizer, which facilitates unfolding and irreversible aggregation of proteins. Since water is a participant in almost all protein degradation pathways, reduction of aqueous protein solution to a dry powder provides an alternative formulation methodology to enhance the stability of protein pharmaceuticals.

**[0006]** One approach to stabilizing proteins is to dry them using various techniques, including freeze-drying, spray-drying, lyophilization, and desiccation. Dried proteins are stored as dry powders until their use is required.

**[0007]** A serious drawback to drying of proteins is that often one would like to use proteins in some sort of flowable form. Parenteral injection and the use of drug delivery devices for sustained delivery of ~~drug~~ drugs are two examples of the applications where one would like to use proteins in a flowable form. For injection, dried proteins must be reconstituted, adding additional steps which are time-consuming and where contamination may occur, and exposing the protein to potentially destabilizing conditions [7]. For drug delivery ~~devices~~ devices, the protein formulations must be stable for extended periods of time at body temperature and maintain their flowability for the expected life of the device.

**[0008]** Solution formulations of proteins/peptides in ~~non-aqueous~~nonaqueous polar aprotic solvents such as DMSO and DMF have been shown to be stable at elevated temperatures for long periods of time [8]. However, such ~~solvent based~~ solvent- based formulations will not be ~~useable~~ usable for all proteins since many proteins have low solubility in these solvents. The lower the solubility of the protein in the formulation, the more solvent would have to be used for delivery of a specific amount of protein. Low concentration solutions may be useful for injections, but may not be useful for ~~long term~~long-term delivery at low flow rates.

**[0009]** Proteins have been formulated for delivery using perfluorodecalin [9, 10], methoxyflurane [9], high concentrations in water [11], polyethylene glycol [12], PLGA [13, 14], ethylenevinylacetate/polyvinylpyrridone mixtures [15], and PEG400/povidone [16]. However, these formulations were not shown to retain a uniform suspension of protein in viscous ~~vehicle~~ vehicles over long periods of time.

**[0010]** Many biologically active compounds degrade over time in aqueous solution. Carriers in which proteins do not dissolve but rather are ~~suspended,~~ suspended can often offer improved chemical stability. Furthermore, it can be beneficial to suspend the beneficial agent in a carrier when the agent exhibits low solubility in the desired vehicle. However, suspensions can

have poor physical stability due to settling and agglomeration of the suspended beneficial agent. The problems with ~~non-aqueous~~nonaqueous carriers tend to be exacerbated as the concentration of the active compound is increased.

**[0011]** Dispersing powdered proteins or peptides in lipid vehicles to yield parenteral sustained release formulations has been investigated [17-21]. The vehicles used were either various vegetable (sesame, soy, peanut, etc.) or synthetic oils (e.g., Miglyol) gelled with aluminum fatty acid esters such as aluminum stearates (mono-, di- or tri-), or with a polyglycerol ester. Although theoretically these vehicles might preclude solution denaturation and protect the drug from aqueous chemical degradation, the vehicles themselves are unstable at higher temperatures. The storage of liquid vegetable oils at body temperatures results in the formation of reactive species such as free fatty acids and peroxides (a process which is accelerated by the presence of traces of various metal ions such as copper or iron which can leach from some implantable devices). These peroxides not only adversely affect protein stability [22] but would be toxic when delivered directly to, for example, the central nervous system of a human or animal.

**[0012]** The sustained delivery of drugs has many advantages. Use of implantable devices assures patient compliance, since the delivery device is ~~tamper-proof~~ tamperproof. With one insertion of a device, rather than daily injections, there is reduced site irritation, fewer occupational hazards for ~~practitioners~~ practitioners, improved cost effectiveness through decreased costs of equipment for repeated injections, reduced hazards of waste disposal, and enhanced efficacy through controlled release as compared with depot injection. The use of implantable devices for sustained delivery of a wide variety of drugs or other beneficial agents is well known in the art. Typical devices are described, for example, in U.S. Patents Nos: 5,034,229; 5,057,318; 5,110,596; and 5,782,396. The disclosure of each of these patents is incorporated herein by reference.

**[0013]** For drug delivering implants, dosing durations of up to one year are not unusual. Beneficial agents which have low therapeutic delivery rates are prime candidates for use in implants. When the device is implanted or stored, settling of the beneficial agent in a liquid formulation can occur. This heterogeneity can adversely affect the concentration of the beneficial agent dispensed. Compounding this problem is the size of the implanted beneficial

agent reservoir. Implant reservoirs are generally on the order of 25-250 μl, but can be up to 25 ml.

**[0014]** Viscous formulations have been prepared using two separate components to be mixed with ~~drug~~ a drug at use [23], thickening agents added to aqueous compositions [24], gelling agents added to aqueous drug solutions [25], porous textile sheet material [26], thickening agents with oleaginous material [27], viscous aqueous ~~carrier~~ carriers for limited solubility ~~drug~~ drugs [28], and extrudable elastic gels [29]. However, these formulations are mixed at use, contain aqueous components, use sheet matrices, or are delivered topically, orally, or intraduodenally.

**[0015]** Stability of formulations can be enhanced by freeze-drying, lyophilizing or spray-drying the active ingredient. The process of drying the active ingredient includes further advantages such as the use of compounds ~~which~~ that are relatively unstable in aqueous solution that can be processed and filled into dosage containers, dried without elevated temperatures, and then stored in the dry state in which there are relatively few stability problems.

**[0016]** Pharmaceutical formulations, particularly parenteral products, should be sterilized after being sealed in the final container and within as short a time as possible after the filling and sealing have been completed. (See, for ~~example~~ example, Remington, Pharmaceutical Sciences, 15th ed. (1975)). Examples of sterilization techniques include thermal or dry-heat, aseptic, and ionized radiation. Combinations of these sterilization procedures may also be used to produce a sterile product.

**[0017]** There is a need to be able to deliver protein compositions to the body which are stable at body temperatures over extended periods of time to enable ~~long-term~~long-term delivery of the protein. There is a need to be able to deliver concentrations of proteins that are efficacious. There is a need for a novel ~~non-aqueous~~nonaqueous formulation capable of homogeneously suspending proteins and dispensing such agents at body temperatures and low flow rates over extended periods of time.

## ~~Summary of the Invention~~

## BRIEF SUMMARY OF THE INVENTION

**[0018]** The present invention provides stable ~~single-phase~~single-phase ~~non-aqueous~~nonaqueous biocompatible viscous vehicles capable of forming uniform suspensions

with proteins. The components of the viscous vehicle comprise at least two of ~~polymer, surfactant, and solvent~~ a polymer, a surfactant, and a solvent. The ratios of the components will vary depending on the molecular weight of the components and the desired viscosity of the final vehicle. Presently preferred component ratios are: polymer, about 5% to about 60%; solvent, about 5% to about 60%; and ~~sufactant~~ surfactant, about 5% to about 40%.

**[0019]** The present invention also provides stable formulations in which beneficial agents are uniformly suspended in stable ~~single phase~~single-phase ~~non-aqueous~~nonaqueous biocompatible viscous vehicles. In particular, the beneficial agents are formulated in the viscous vehicles at concentrations of at least about 0.1%, depending upon the potency of the beneficial agent. These stable formulations may be stored at the temperature appropriate for the beneficial agent, ranging from ~~cold,~~ cold to body temperature (about 37°C) for long periods of time (1 month to 1 year or more). In a preferred ~~embodiment~~ embodiment, the formulation comprises about 0.1 to 50% (w/w) of beneficial agent, depending on the potency of the beneficial agent, the duration of treatment, and the rate of release for the drug delivery system.

**[0020]** These formulations are especially useful in implantable delivery devices for ~~long term~~long-term delivery (e.g., 1 to 12 months or longer) of beneficial agent at body temperature, preferably about 37°C. Thus, the present invention also provides for the delivery of ~~said~~ such proteins to the body ~~over~~ over an extended period of time to enable ~~long term~~long-term delivery of the protein at low flow rates of about 0.3 to 100 $\mu$l/day, preferably about 0.3 to 4 $\mu$l/day for about a ~~6 month~~ six-month delivery period and preferably 5 to 8 $\mu$l/day for about a ~~3 month~~ three-month delivery period.

**[0021]** In another aspect, the invention provides methods for preparing stable ~~non-aqueous~~nonaqueous biocompatible formulations of a beneficial agent in a ~~single phase~~single-phase viscous vehicle. Preferred formulations comprise about 0.1 to 50% (w/w) beneficial agent depending on the potency of the beneficial agent, the duration of treatment, and the rate of release from the delivery system.

**[0022]** In yet a further aspect, the invention provides methods for treating a subject suffering from a condition which may be alleviated by administration of a beneficial agent, ~~said~~ these methods comprising administering to ~~said~~ the subject an effective amount of a stable ~~non-aqueous~~nonaqueous formulation comprising at least one beneficial agent uniformly suspended in a ~~single phase~~single-phase viscous vehicle.

**[0023]** A further aspect of the invention is that ~~non-aqueous~~nonaqueous ~~single phase~~single-phase viscous vehicles containing beneficial agents are chemically and physically stable over a broad temperature range for long periods of time. The beneficial agents in the viscous vehicles are also chemically and physically stable over a broad temperature range for long periods of time. Thus, these formulations are advantageous in that they may be shipped and stored at temperatures below, at, or above room temperature for long ~~period~~ periods of time. They are also suitable for use in implantable delivery devices in which the formulation must be stable at body temperature for extended periods of time.

**[0024]** The formulations of the present invention also remain stable when delivered from implantable drug delivery systems. The beneficial agents have been shown to exhibit zero order release rates when delivered from implantable drug delivery systems at very low flow rates over extended periods of time.

## ~~Brief Description of the Drawings~~

## BRIEF DESCRIPTION OF THE SEVERAL VIEWS OF THE DRAWINGS

**[0025]** Figure 1 shows the stability of ~~hGH~~ human growth hormone (hGH) formulations of the present invention as determined at ~~37°C~~ 37° C by reverse phase HPLC.

**[0026]** Figure 2 shows the stability of hGH formulations of the present invention as determined at ~~37°C~~ 37° C by size exclusion chromatography.

**[0027]** Figure 3 shows the average release rate (μl/day) of 10% (w/w) spray-dried lysozyme in formulations of the present invention.

**[0028]** Figure 4 shows the average release rate (μl/day) of 10% (w/w) spray-dried hGH in a glycerol monolaurate/lauryl lactate/polyvinylpyrrolidone vehicle.

**[0029]** Figure 5 shows the average release rate (μg/day) of 10% lysozyme in a lauryl alcohol/polyvinylpyrrolidone vehicle.

**[0030]** Figure 6 shows the average release rate ~~((μg/day)~~ (μg/day) of 25% lysozyme in a glycerol monolaurate/lauryl lactate/polyvinylpyrrolidone vehicle.

**[0031]** Figure 7 shows the average release rate ~~((μg/day)~~ (μg/day) of 33% lysozyme in a glycerol monolaurate/lauryl lactate/polyvinylpyrrolidone vehicle.

**[0032]** Figure 8 shows the average release rate ~~((μg/day)~~ μg/day) of 45% lysozyme in a glycerol monolaurate/lauryl lactate/polyvinylpyrrolidone vehicle.

~~Detailed Description of the Invention~~

DETAILED DESCRIPTION OF THE INVENTION

**[0033]** The present invention is drawn to the unexpected discovery that uniformly suspending beneficial agents in ~~non-aqueous~~nonaqueous ~~single phase~~single-phase biocompatible viscous vehicles results in stable formulations which can be delivered at body temperature over an extended period of time at low flow rates. Previously known formulations of beneficial agents which are buffered aqueous or ~~non-aqueous~~nonaqueous solutions which may or may not contain excipients ~~do not provide~~ are not formulations which can be uniformly dispensed at body temperatures at low flow rates over an extended period of time without exhibiting unacceptable amounts of aggregation or degradation of the formulation. The presently claimed formulations stabilize beneficial agents and can be stored at the temperature appropriate for the beneficial agent. The temperatures can range from cold (not exceeding 8°C) to body temperature (about 37°C) for long periods of time. These formulations are especially useful in implantable delivery devices for ~~long term~~long-term delivery (e.g., 1 to 12 months or longer) of ~~drug~~ a drug at low flow rates and at body temperature, preferably about 37°C.

**[0034]** Standard beneficial agent formulations consist of dilute aqueous or ~~non-aqueous~~nonaqueous solutions or suspensions. Drug stability is usually achieved by varying one or more of the following: pH, buffer type, ionic strength, excipients (EDTA, ascorbic acid, etc.) For these formulations, degradation pathways requiring water (hydrolysis, deamidation, racemization) cannot be fully stabilized. In the present invention, beneficial agents formulated in ~~non-aqueous~~nonaqueous biocompatible ~~single phase~~single-phase viscous vehicles ~~containing~~ containing, for example, polyvinylpyrrolidone, vinyl acetate, and/or polyoxyethylenepolyoxypropylene block copolymers were shown to be chemically and physically stable. The viscosity of the formulation will depend upon a number of criteria, including the beneficial agent potency and concentration, and the process by which the formulation is prepared. The viscosity of the formulation can be chosen so that the desired amount of beneficial agent is delivered over the desired period of time.

**[0035]** The invention also consists of ~~non-aqueous~~nonaqueous ~~single phase~~single-phase biocompatible viscous vehicles capable of uniformly suspending beneficial agents and formulations containing at least one beneficial agent uniformly suspended in ~~said~~ the viscous

vehicle. The invention also consists of formulations containing at least one beneficial agent uniformly suspended in a ~~non-aqueous~~nonaqueous ~~single-phase~~single-phase biocompatible viscous vehicle, which formulations are stable for an extended period of time at body ~~temperatures,~~ temperature and capable of delivering ~~said~~ the beneficial agents uniformly at low flow rates. The discovery consists of the realization that stable ~~non-aqueous~~nonaqueous viscous vehicles improve the stability of beneficial agents in a wide range of formulation conditions including concentration, elevated temperatures and duration of stable formulation, thus making possible the delivery of beneficial agents in ~~long-term~~long-term implantable devices that would not otherwise be feasible.

~~Definitions~~

DEFINITIONS

**[0036]** As used herein, the following terms have the following meanings:

The term "chemical stability" means that an acceptable percentage of degradation products produced by chemical pathways such as oxidation, deamidation, or hydrolysis is formed. In particular, a formulation is considered chemically stable if no more than about 35% breakdown products are formed after 2 months at 37°C.

**[0037]** The term "physical stability" means that an acceptable percentage of aggregates (e.g., dimers, trimers and larger forms) are formed by the beneficial agent. For the formulation (viscous vehicle and beneficial ~~agent)~~ agent), this term means that the formulation retains stability, flowability, and the ability to uniformly dispense the beneficial agent. In particular, a formulation is considered physically stable if no more than about 15% aggregates are formed after two months at 37°C.

**[0038]** The term "stable formulation" means that at least about 65% chemically and physically stable beneficial agent remains after two months at 37°C (or equivalent conditions at an elevated temperature). Particularly preferred formulations are those which retain at least about 80% chemically and physically stable beneficial agent under these conditions. Especially preferred stable formulations are those which do not exhibit degradation after sterilizing irradiation (e.g., gamma, beta or electron beam).

**[0039]** The term "beneficial agent" means peptides, proteins, nucleotides, hormones, viruses, antibodies, etc. that comprise polymers of amino acid or nucleic acid residues. These

beneficial agents are generally degradable in water and generally stable as a dry powder at elevated temperatures. Synthetically produced, naturally derived or recombinantly produced moieties are included in this term. The term also includes lipoproteins and ~~post-translationally~~ post-translationally modified forms, e.g., glycosylated proteins. Analogs, derivatives, agonists, antagonists and pharmaceutically acceptable salts of any of these are included in this term. The term also includes proteins and/or protein substances which have D-amino acids, modified, derivatized or non-naturally occurring amino acids in the D- or L- configuration and/or peptomimetic units as part of their structure. The term ~~protein~~ "protein" will be used in the present invention. The term also means that the beneficial agent is present in the solid state, e.g., powder or crystalline.

**[0040]** The term "excipient" means a more or less inert substance in a formulation that is added as a diluent or vehicle or to give form or consistency. Excipients are distinguished from solvents such as ETOH, which are used to dissolve drugs in formulations. Excipients include non-ionic surfactants such as polysorbates, which are used to solubilize drugs in formulations; preservatives such as benzyl alcohols or methyl or propyl parabens, which are used to prevent or inhibit microbial growth; chelating agents; flavoring agents; and other pharmaceutically acceptable formulation aides.

**[0041]** The term "viscous vehicle" means a vehicle with a viscosity in the range of about 1,000 to 10,000,000 poise. The term includes Newtonian and non-Newtonian materials. Preferred are vehicles with a viscosity of about 10,000 to 250,000 poise. The formulations of this invention can uniformly expel beneficial agents suspended in the viscous vehicle from implantable drug delivery devices. The formulations exhibit a shear rate at the exit of ~~said~~ the devices of 1 to 1 x $10^{-7}$ reciprocal second, preferably an exit shear rate of 1 x $10^{-2}$ to 1 x $10^{-5}$ reciprocal second.

**[0042]** The term "~~single phase~~single-phase" means a solid, ~~semi-solid~~semisolid, or liquid homogeneous system that is both physically and chemically uniform throughout as determined by differential scanning calorimetry (DSC). The DSC scan should show one peak indicative of a ~~single phase~~single-phase.

**[0043]** The term "biocompatible" means a property or characteristic of a viscous vehicle to disintegrate or break down, over a prolonged period of time, in response to the biological environment in the patient, by one or more physical or chemical degradative

processes, for ~~example~~ example, by enzymatic action, oxidation or reduction, hydrolysis (proteolysis), displacement, ~~e.g.~~ e.g., ion exchange, or dissolution by solubilization, emulsion or micelle formation, and which material is then absorbed by the body and surrounding tissue, or otherwise dissipated thereby.

**[0044]** The term "polymer" includes polyesters such as PLA (polylactic acid) ~~[having~~ having an inherent viscosity in the range of about 0.5 to ~~2.0 i.v.]~~ 2.0 i.v. and PLGA (polylacticpolyglycolic acid) ~~[having~~ having an inherent viscosity in the range of about 0.5 to ~~2.0 i.v.]~~, 2.0 i.v., pyrrolidones such as polyvinylpyrrolidone (having a molecular weight range of about 2,000 to 1,000,000), esters or ethers of unsaturated alcohols such as vinyl acetate, and polyoxyethylenepolyoxypropylene block copolymers (exhibiting a high viscosity at 37°C) such as Pluronic 105. ~~Currently~~ A currently preferred polymer is polyvinylpyrrolidone.

**[0045]** The term "solvent" includes carboxylic acid esters such as lauryl lactate, polyhydric alcohols such as glycerin, polymers of polyhydric alcohols such as polyethylene glycol (having a molecular weight of about 200 to 600), fatty acids such as oleic acid and octanoic acid, oils such as castor oil, propylene carbonate, lauryl alcohol, or esters of polyhydric alcohols such as triacetin acetate. Currently preferred is lauryl lactate.

**[0046]** The term "surfactant" includes esters of polyhydric alcohols such as glycerol monolaurate, ethoxylated castor oil, polysorbates (for ~~example~~ example, Polysorbate 80), esters or ethers of saturated alcohols such as myristyl lactate (Ceraphyl 50), and polyoxyethylenepolyoxypropylene block copolymers such as Pluronic (for example, F68). Currently preferred are gylcerol monolaurate and polysorbates.

**[0047]** The term "antioxidant" means a pharmaceutically acceptable aid for ~~stablization~~ stabilization of the beneficial agent against degradation such as oxidation. Antioxidants include, but are not limited to, tocopherol (vitamin E), ascorbic acid, ascorbyl palmitate, butylated hydroxyanisole, butylated hydroxytoluene, and propyl gallate. A preferred antioxidant depends on solubility and the efficiency of the antioxidant for protecting against degradation or chemical change of the beneficial agent in the preferred vehicle. Currently preferred is ascorbyl palmitate.

~~Preparation of Formulations~~

PREPARATION OF FORMULATIONS

**[0048]** The present invention is drawn to stable ~~non-aqueous~~nonaqueous ~~single phase~~single-phase biocompatible viscous vehicles capable of suspending beneficial agents and uniformly dispensing ~~said~~ the beneficial agents at body ~~temperatures~~ temperature at low flow rates over an extended period of time. The present invention is also directed to formulations containing beneficial agents uniformly suspended in ~~said~~ the ~~single phase~~single-phase biocompatible viscous vehicles which are stable for prolonged periods of time at body ~~temperatures~~temperature.

**[0049]** Examples of beneficial agents that may be formulated using the present invention include those peptides or proteins that have biological activity or that may be used to treat a disease or other pathological condition. They include, but are not limited to, adrenocorticotropic hormone, angiotensin I and II, atrial natriuretic peptide, bombesin, bradykinin, calcitonin, cerebellin, dynorphin N, alpha and beta endorphin, endothelin, enkephalin, epidermal growth factor, fertirelin, follicular gonadotropin releasing peptide, galanin, glucagon, GLP-1, gonadorelin, gonadotropin, goserelin, growth hormone releasing peptide, histrelin, human growth hormone, insulin, interferons, leuprolide, LHRH, motilin, nafarerlin, neurotensin, oxytocin, relaxin, somatostatin, substance P, tumor necrosis factor, triptorelin, vasopressin, growth hormone, ~~nerve.growth~~ nerve growth factor, blood clotting factors, ribozymes, and antisense oligonucleotides. Analogs, derivatives, ~~antagonists~~ agonists and pharmaceutically acceptable salts of the above may also be used.

**[0050]** The beneficial agents useful in the formulations and methods of the present invention can be used in the form of a salt, preferably a pharmaceutically acceptable salt. Useful salts are known to those of skill in the art and include salts with inorganic acids, organic acids, inorganic bases, or organic bases.

**[0051]** Beneficial agents that are not readily soluble in ~~non-aqueous~~nonaqueous solvents are preferred for use in the present invention. One of skill in the art can easily determine which compounds will be useful on the basis of their solubility. The amount of beneficial agent may vary depending on the potency of the compound, the condition to be treated, the solubility of the compound, the expected dose and the duration of administration.

(See, for example, ~~Gilman, et. al,~~ Gilman et al., The Pharmacological Basis of Therapeutics, 7th ed. (1990) and Remington, Pharmacological Sciences, 18th ed. (1990), the disclosures of which are incorporated herein by reference.)

**[0052]** It has been unexpectedly found that using a stable ~~non-aqueous~~nonaqueous ~~single phase~~single-phase biocompatible viscous vehicle increases the stability of the beneficial agent. For example, as seen in Figures 1 and 2, human growth hormone (hGH) was found to be stable at 37°C over 12 weeks in formulations of polyvinylpyrrolidone/PEG; Pluronic; and glycerol monolaurate/lauryl lactate/polyvinylpyrrolidone. Figure 1 shows stability results using reverse phase HPLC. Figure 2 shows stability results using size exclusion chromatography.

**[0053]** Generally, stable ~~non-aqueous~~nonaqueous ~~single phase~~single-phase ~~biocompatiible~~ biocompatible viscous vehicles may be prepared by combining the dry (low moisture content) ingredients in a dry box or under other dry conditions and blending them at elevated temperature, preferably about 40 to about 70°C, to allow them to liquify. The liquid vehicle is allowed to cool to room temperature. Differential scanning calorimetry was used to verify that the vehicle was ~~single phase~~single-phase. The final moisture content of the viscous vehicle was <2%.

**[0054]** Generally, the stable formulations of the present invention may be prepared by combining the vehicle and beneficial agent under dry conditions and blending them under vacuum at elevated temperature, preferably about 40 to about 70°C, to disperse the beneficial agent uniformly throughout the vehicle. The formulation is allowed to cool to room temperature.

**[0055]** It has been found that drying the beneficial agent prior to formulation enhances the stability of the formulation.

**[0056]** It has also been found that adding antioxidants, such as tocopherol, ascorbic acid, ascorbyl palmitate, butylated hydroxyanisole, butylated hydroxytoluene, and propyl gallate reduces the formation of degradation products (e.g., unstable chemical intermediates) during sterilization.

Methodology

**[0057]** We have found that stable ~~non-aqueous~~nonaqueous beneficial agent formulations utilizing viscous vehicles may be prepared by combining the ingredients for the viscous vehicle under dry conditions and blending them at elevated temperature to allow them to

liquify and form a ~~single phase~~single-phase. Once a ~~single phase~~single-phase viscous vehicle is formed, the vehicle is allowed to cool to room temperature. ~~Beneficial~~ A beneficial agent is added with mixing at elevated temperature under vacuum to uniformly disperse it in the viscous vehicle.

**[0058]** We have tested these beneficial agent formulations, for ~~example~~ example, formulations of hGH, for stability by subjecting them to accelerated aging tests. Results show that these formulations remained stable over extended periods of time.

**[0059]** We have tested beneficial agent formulations, for ~~example~~ example, human growth hormone and lysozyme, for stability by suspending them in a variety of ~~non-aqueous~~nonaqueous ~~single phase~~single-phase viscous vehicles prepared according to the present invention, then subjecting them to accelerated aging at elevated temperatures. The stability of the formulations was measured. Results of these studies demonstrate that these formulations were stable at conditions that approximate or exceed storage for one year at 37°C.

**[0060]** We have also tested beneficial agent formulations prepared as described herein for stability after 2.5 megarads gamma irradiation. Results show that these formulations remained chemically and physically stable after such irradiation.

~~Methods~~

METHODS

**[0061]** The following methods were used to perform the studies in the ~~Examples~~ examples that follow.

~~1. Preparing Protein powders~~

1. Preparing Protein Powders

Human Growth Hormone (~~obtained~~ obtained, for example, from BresaGen Limited, Adelaide, Australia)

**[0062]** The active agent was reconstituted in deionized water. The solution containing the active agent was buffer exchanged using an Amicon Diaflo® Ultrafiltration membrane (molecular weight cut-off 10,000).

**[0063]** The diafiltrated active agent solution was spray dried using a Yamato mini-spray dryer. Powder was collected in a collection vessel through a cyclone trap. All handling of the ~~spray dried~~ spray-dried powder took place in a dry box evacuated with nitrogen. The

generated powder was analyzed for particle size and distribution, moisture content, protein content and stability by size exclusion and reverse-phase chromatography.

**[0064]** It is known that the conformation of some proteins can be stabilized by the addition of a sugar (such as sucrose or mannitol) or a polyol (such as ethylene glycol, glycerol, glucose, and dextran.)

~~2. Preparation of Viscous Vehicles~~

2. Preparation of Viscous Vehicles

**[0065]** We have found that stable ~~single phase~~single-phase biocompatible viscous vehicles may be prepared by combining the ingredients and blending them at elevated temperatures to allow them to liquify and form a ~~single phase~~single-phase. A differential scanning calorimetry scan showed one peak, indicative of a ~~single phase~~single-phase. The mixing was completed under vacuum to remove trapped air bubbles produced from the powders. The mixer was a dual helix blade mixer (D.I.T.) which runs at a speed around 40 rpm. Higher speeds can be used but are not required.

**[0066]** If a ~~three component~~ three-component viscous vehicle ~~is~~ was prepared, the solvent portion of the vehicle was added to the heated bowl of the mixer first, followed by the surfactant. The polymer was added last, and the ingredients were mixed until a solution (~~single phase~~single-phase) resulted. Vacuum was applied during mixing to remove air bubbles. The solution was dispensed from the bowl while at elevated ~~temperature,~~ temperature and allowed to cool to room temperature. On ~~cooling~~ cooling, the vehicle exhibited increased viscosity. ~~Two~~ Two- and ~~single~~ single-component gels were made using the same process.

~~3. Preparation of beneficial agent formulations~~

3. Preparation of Beneficial Agent Formulations

**[0067]** To prepare the formulation, the ~~single phase~~single-phase viscous vehicle was heated and then blended under vacuum with a weighed amount of beneficial agent. The beneficial agent and the ~~single phase~~single-phase viscous vehicle were blended in the same manner as the vehicle was prepared, using a dual helix blade mixer (or other similar ~~mixer).~~ ~~Mixing~~ mixer), at a speed ~~was~~ between 40 and 120 rpm for approximately 15 minutes or until a

uniform dispersion was attained. The resulting mixture was removed from the mixer, sealed in a dry container, and allowed to cool to room temperature.

~~4. Preparation of reservoirs~~

4. Preparation of Reservoirs

**[0068]** The reservoirs of implantable drug delivery devices (as disclosed in U.S. Patent Application Serial No. 08/595,761, incorporated herein by reference) were filled with the appropriate hGH formulation. The formulation was filled into titanium reservoirs with a polymer plug blocking each end. The filled reservoir was then sealed in a polyfoil bag and placed in a stability testing oven.

**[0069]** It should be noted that the formulations in the reservoirs of these devices are completely isolated from the outside environment.

~~5. Reverse Phase-HPLC (RP-HPLC)~~

5. Reverse Phase-HPLC (RP-HPLC)

**[0070]** All stability samples of hGH were assayed for protein content and chemical stability by reverse phase chromatography (RP-HPLC). Analyses were performed on a Hewlett Packard HP-1090 system with a refrigerated autosampler (4°C). The chromatographic conditions used are listed below.

**[0071]** TABLE 1

~~RP-HPLC Chromatographic Conditions~~

RP-HPLC Chromatographic Conditions

| Description | Parameter |
|---|---|
| Column | J.T. Baker-C18, 4.6x250 mm |
| Flow Rate | 1.0 mL/min |
| Detection | 214 nm |
| Mobile Phase | A: 0.1% TFA in water |
| | B: 0.1% TFA in acetonitrile |

| Gradient | time | %A | %B |
|---|---|---|---|
| | 0 | 65 | 35 |
| | 5 | 50 | 50 |
| | 45 | 35 | 65 |
| | 50 | 30 | 70 |
| | 55 | 65 | 35 |

**[0072]** An hGH reference standard solution was prepared and its protein content calculated from the absorbance measurement at 280 nm. Three dilutions of this solution, representing 80%, 100%, and 120% of the expected concentration of hGH in the ~~samples~~ samples, were run in duplicate at the beginning and the end of each run and used to calculate the total protein content of the samples.

~~6. Size Exclusion Chromatography (SEC)~~

6. Size Exclusion Chromatography (SEC)

**[0073]** All stability samples of hGH were assayed for protein content and ~~high molecular weight~~ high-molecular-weight degradation products by size exclusion chromatography. Analyses were performed on a Hewlett Packard HP-1090 system with a refrigerated autosampler (4°C). The chromatographic conditions used are listed below

**[0074]** TABLE 2

SEC Chromatographic Conditions

| Description | Parameter |
|---|---|
| Column | TSK-2000SWXL |
| Flow Rate | 0.5 ml/~~min~~min. |
| Detection | ~~214nm~~214 nm |
| Mobile Phase | 25 mM sodium phosphate, 100 mM sodium chloride, pH 7.0 |

**[0075]** ~~A~~ An hGH reference standard solution was prepared and its protein content calculated from the absorbance measurement at 280 nm. Three dilutions of this solution, representing 80%, 100%, and 120% of the expected concentration of hGH in the ~~samples~~ samples, were run in duplicate at the beginning and the end of each run and used to calculate the total protein content of the samples. The amount of ~~high molecular weight~~ high-molecular-weight degradation products was calculated by area normalization.

**[0076]** The following examples are offered to illustrate this invention and are not meant to be construed in any way as limiting the scope of this invention.

## EXAMPLE 1

### ~~Preparation of Non-aqueous Single Phase Viscous Vehicles~~

### Preparation of Nonaqueous Single-Phase Viscous Vehicles

**[0077]** The ~~non-aqueous~~nonaqueous ~~single phase~~single-phase viscous vehicles can be prepared as follows and shown in the ~~below table~~table below.

**[0078]** A. Glycerol monolaurate (Danisco Ingredients, New Century, ~~Kansas~~KS) (25 g) was dissolved in lauryl lactate (ISP Van Dyk Inc., Belleville, NJ) (35 g) at 65°C. Polyvinylpyrrolidone C30 (BASF, Mount Olive, NJ) (40 g) was added and the mixture blended at about 40 rpm in a dual helix blade mixer (D.I.T.) until a ~~single phase~~single-phase was achieved. Trapped air bubbles were removed by applying vacuum to the mixing chamber. The ~~single phase~~single-phase vehicle was dispensed from the ~~mixer,~~ mixer and allowed to cool to room temperature.

**[0079]** B. Glycerol monolaurate (Danisco Ingredients, New Century, ~~Kansas~~KS) (25 g) was dissolved in lauryl lactate (ISP Van Dyk Inc., Belleville, NJ) (35 g) at 65°C. Polyvinylpyrrolidone C17 (BASF, Mount Olive, NJ) (40 g) was added and the mixture blended at about 40 rpm in a dual helix blade mixer (D.I.T.) until a ~~single phase~~single-phase was achieved. Trapped air bubbles were removed by applying vacuum to the mixing chamber. The ~~single phase~~single-phase vehicle was dispensed from the ~~mixer,~~ mixer and allowed to cool to room temperature.

**[0080]** C. Polyvinylpyrrolidone C30 (BASF, Mount Olive, NJ) (50 g) was dissolved in polyethylene glycol 400 (Union Carbide) (50 g) at approximately 65°C until a ~~single~~

~~phase~~single-phase solution was formed. The ~~single phase~~single-phase vehicle was dispensed from the ~~mixer,~~ mixer and allowed to cool to room temperature.

**[0081]** D. Polyvinylpyrrolidone C17 (BASF, Mount Olive, NJ) (50 g) was dissolved in polyethylene glycol 400 (Union Carbide) (50g) at approximately 65°C until a ~~single phase~~single-phase solution was formed. The ~~single phase~~single-phase vehicle was dispensed from the ~~mixer,~~ mixer and allowed to cool to room temperature.

**[0082]** E. Polyvinylpyrrolidone C17 (BASF, Mount Olive, NJ) (50 g) was dissolved in castor oil (Spectrum, Gardena, CA) (50 g) at approximately 65°C until a ~~single phase~~single-phase solution was formed. The ~~single phase~~single-phase vehicle was dispensed from the ~~mixer,~~ mixer and allowed to cool to room temperature.

**[0083]** F. Polyvinylpyrrolidone C17 (BASF, Mount Olive, NJ) (50 g) was dissolved in octanoic acid (Spectrum, Gardena, CA) at approximately 65°C until a ~~single phase~~single-phase solution was formed. The ~~single phase~~single-phase vehicle was dispensed from the ~~mixer,~~ mixer and allowed to cool to room temperature.

**[0084]** G. Polyvinylpyrrolidone C17 (BASF, Mount Olive, NJ) (50 g) was dissolved in oleic acid (Spectrum, Gardena, CA) at approximately 65°C until a ~~single phase~~single-phase solution was formed. The ~~single phase~~single-phase vehicle was dispensed from the ~~mixer,~~ mixer and allowed to cool to room temperature.

**[0085]** H. Polyvinylpyrrolidone C17 (BASF, Mount Olive, NJ) (35%) was dissolved in glycerin (Baker, NJ) (65%) at approximately 65°C until a ~~single phase~~single-phase solution was formed. The ~~single phase~~single-phase vehicle was dispensed from the ~~mixer,~~ mixer and allowed to cool to room temperature.

**[0086]** I. Cremophor EL (ethoxylated castor oil) (BASF, Mount Olive, NJ) (5%) was dissolved in castor oil (Spectrum, Gardena, CA) (70%), and polyvinylpyrrolidone C17 (BASF, Mount Olive, NJ) (25%) was added and dissolved by mixing at approximately 40 rpm to form a ~~single phase~~single-phase vehicle. The ~~single phase~~single-phase vehicle was dispensed from the ~~mixer,~~ mixer and allowed to cool to room temperature.

**[0087]** J. Pluronic 105 (BASF, Mount Olive, NJ) was heated to approximately 65°C with mixing until melted. The ~~single phase~~single-phase vehicle was dispensed from the ~~mixer,~~ mixer and allowed to cool to room temperature.

**[0088]** K. Pluronic F68 (BASF, Sigma) 10% w/w and butylhydroxytoluene (Spectrum) 1% w/w were dissolved in 49% w/w propylene carbonate (Aldrich) by mixing under vacuum at 60°C until the materials dissolved. The vacuum was released, and the resulting liquid was added to 40% w/w ~~poly lactic~~ polylactic acid ~~[poly~~(poly(D,L-lactide), Resomer R207, Boehringer ~~Ingelheim]~~ Ingelheim). All components were mixed by hand at 60°C using a spatula until the ~~poly lactic~~ polylactic acid was dissolved to form a ~~single phase~~single-phase vehicle. The ~~single phase~~single-phase vehicle was moved to a vacuum chamber to remove remaining air bubbles and allowed to cool to room temperature.

**[0089]** L. Myristyl lactate (Ceraphyl 50, ISP Van Dyk) 20% w/w was dissolved in 25~~%~~ percent w/w lauryl alcohol (Sigma) under vacuum at 60°C ~~until the material dissolved~~. The vacuum was released, and the resulting liquid was added to a mixing bowl. ~~55%~~ Fifty-five percent w/w ~~Polyvinylpyrrolidone~~ polyvinylpyrrolidone (BASF, 17pf) was added on top and the contents of the bowl were mixed at 40 rpm at 60°C under vacuum until all components were miscible and formed a ~~single phase~~single-phase vehicle. Vacuum was applied until air bubbles were removed from the ~~single phase~~single-phase vehicle.

**[0090]** Table 3

Component Ratios

| Polymer | Component Surfactant | Solvent | Ratio | Viscosity at Low Shear Rate (Poise) |
|---|---|---|---|---|
| PVP | GML | LL | 53:5:42 | 25,000 |
| PVP | GML | LL | 55:10:35 | 50,000 |
| PVP | GML | LL | 50:15:35 | 7,000 |
| PVP | ----- | LA | 60:40 | |
| PVP | Ceraphyl 50 | LA | 60:10:30 | |
| PVP | ----- | oleic acid | 50:50 | 30,000 |
| PVP | ----- | octanoic acid | 55:45 | ~~7.000~~7,000 |
| PVP | polysorbate 80 | ----- | 50:50 | |
| PVP | ----- | PEG 400 | 50:50 | |
| PVP | caster oil | ----- | 50:50 | |
| ----- | Pluronic 105 | ----- | 100 | 1,000,000 |
| PVP | ----- | glycerin | 50:50 | 5,000 |

| | | | |
|---|---|---|---|
| PLA | F68 | PC | 30:10:60* |
| PVP (C17) | ML | LA | 50:25:25 |
| PVP (C17) | polysorbate 80 | LL | 55:40:5 |

**[0091]** Wherein:

GML = glycerol monolaurate

LL = lauryl lactate

PVP = polyvinylpyrrolidine C30

LA = lauryl alcohol

PEG = polyethyleneglycol 400

F68 = poly(propylene oxide)/poly(ethylene oxide) block copolymer (a ~~Member~~ member of the Pluronic family)

PC = propylene carbonate

PLA = ~~poly-lactic~~ polylactic acid

ML = myristyl lactate

* also contains 1% butylhydroxytoluene

## EXAMPLE 2

### ~~Preparation of hGH~~

### Preparation of hGH

A. Preparation by ~~spray-drying~~Spray Drying

**[0092]** Lyophilized hGH (BresaGen Limited, Adelaide, Australia) was reconstituted in 150 ml of deionized water. This stock solution contained 1050 mg of hGH. Buffer exchange was accomplished using an Amicon Diaflo® Ultrafiltration membrane (molecular weight cut-off 10,000). The ultrafiltration cell was connected to an ~~auxilliary~~auxiliary reservoir containing 5mM phosphate buffer (pH 7). The cell's fluid volume, as well as the hGH concentration, remained constant as excipients were replaced by phosphate buffer.

**[0093]** The diafiltrated protein solution (protein concentration in the solution approximately 2%) was spray dried using a Yamato mini-spray dryer. Settings on the spray dryer were as follows: aspiration pressure constantly adjusted to 1.3 kgf/cm$^2$, inlet temperature

120°C, solution flow rate 2.5 (approximately 3 ml/min). Powder was collected in a collection vessel through a cyclone trap. All handling of the ~~spray-dried~~ spray-dried powder took place in a dry box evacuated with nitrogen (% RH: 1-4%). The water content of the suspending vehicles is shown in the below table.

**[0094]** TABLE 4

WATER CONTENT OF SUSPENDING VEHICLES

| Vehicle | Water Content of Vehicle at T 0 % w/w | Water Content of Vehicle in 12 wks. ~~At 37°C~~at 37° C %w/w |
|---|---|---|
| Pluronic 105 | 0.25 | 0.4 |
| GML/LL/PVP | 1.5 | 1.3 |
| PVP/PEG | 2.0 | 2.0 |

**[0095]** Wherein:

GML = glycerol monolaurate

LL = lauryl lactate

PVP = polyvinylpyrrolidine C30

PEG = polyethyleneglycol 400

EXAMPLE 3

Preparation of hGH Formulation

**[0096]** A portion of the ~~single phase~~single-phase viscous vehicle was weighed (9 g) and heated to 60°C. hGH (BresaGen Limited, Adelaide, Australia) (1 g) was added to the vehicle and mixed for 15 minutes. The mixing was completed under vacuum to remove air bubbles added from the powder.

**[0097]** Approximately 10 mg of the spray-dried hGH powder were weighed out (content of hGH in the powder was recalculated based on the determined water and salt content) and mixed with 100 $\mu$l of the vehicle at 55-65°C (3 samples per each vehicle). Special care was

taken while mixing powder in the suspending vehicle to achieve maximum particle uniform dispersion in the vehicle. All steps were done in a dry box.

**[0098]** The resulting suspension was dissolved with 10 ml of release rate buffer and analyzed by size exclusion and reverse-phase chromatography. Spray dried hGH powder was used as a control.

**[0099]** TABLE 5

STABILITY OF hGH SUSPENSIONS AT 37°C AS MEASURED BY SIZE EXCLUSION CHROMATOGRAPHY

| Time Weeks | Spray-dried Powder -80°C %LS | PVP/PEG 400 Suspension %LS | GML/LL/PVP Suspension %LS | Pluronic 105 Suspension %LS |
|---|---|---|---|---|
| 0 | 96±1 | 88±6 | 92±2 | 87±7 |
| 1 | 99±8 | 81±2 | 94±3 | 93±3 |
| 2 | 99±3 | 83±1 | 97±1 | 94±1 |
| 3 | 97±1 | 84±2 | 95±2 | 95±3 |
| 4 | 95±2 | 82±8 | 94±4 | 93±5 |
| 7 | 95±4 | 76±3 | 93±4 | 88±2 |
| 12 | 97±4 | 79±3 | 97±1 | 95±6 |

Each data point represents the mean ± relative standard deviation of three individual samples taken from three separate vials.

**[00100]** TABLE 6

STABILITY OF hGH SUSPENSIONS at 37°C AS MEASURED BY REVERSE PHASE CHROMATOGRAPHY

| Time Weeks | ~~spray~~Spray-dried Powder -80°C %LS | PVP/PEG 400 Suspension %LS | GML/LL/PVP Suspension %LS | Pluronic 105 Suspension %LS |
|---|---|---|---|---|
| 0 | 104±1 | 99±3 | 99±2 | 89±7 |
| 1 | 104±8 | 78±2 | 98±3 | 96±6 |

| | | | | |
|---|---|---|---|---|
| 2 | 104±4 | 73±3 | 95±1 | 96±1 |
| 3 | 104±2 | 78±4 | 97±3 | 97±4 |
| 4 | 100±2 | 74±10 | 93±4 | 96±4 |
| 7 | 108±5 | 72±4 | 96±2 | 94±2 |
| 9 | 102±3 | 66±3 | 92±3 | 93±2 |
| 12 | 101±2 | 66±1 | 89±2 | 92±5 |

Each data point represents the mean ± relative standard deviation of three individual samples taken from three separate vials.

EXAMPLE 4

Preparation of Reservoirs

Release Rate Profiles

**[00101]** Titanium reservoir systems of implantable drug delivery devices (as disclosed in U.S. Patent Application Serial No. 08/595,761, incorporated herein by reference) were each assembled with an osmotic engine, piston, and ~~rate-controlling~~ rate-controlling membrane. The reservoirs were filled with the appropriate amount of viscous vehicle formulation and capped with a flow plug. The systems were placed in a water bath at ~~37°C,~~ 37° C and allowed to release formulation for an extended period of time. Released material was sampled twice per week. Assays for released material were completed using reverse phase HPLC. The resulting concentrations of beneficial agent for each system were converted to the released amount per day. The beneficial agent was found to have a zero order release from the implantable drug delivery ~~device. As~~ device, as shown in Figures 3 through 8.

EXAMPLE 5

Stability of hGH in ~~Non-aqueous~~Nonaqueous Viscous Vehicle Formulations

**[00102]** Formulations of 10% w/w hGH in vehicle were prepared as described above and placed in vials. The formulations were subjected to accelerated aging by storing them at

elevated temperatures and times ~~shown in the below table~~ in a controlled temperature oven shown in the table below.

**[00103]** TABLE 7

| Vehicle | Time(hrs) | Temperature | %LS by SEC | %LS by RP-HPLC |
|---|---|---|---|---|
| Pluronic 105 | 0 | 50°C | 98±3 | 101±3 |
| Pluronic 105 | 1 | 50°C | 98±3 | 101±4 |
| Pluronic 105 | 2 | 50°C | 100±1 | 102±3 |
| Pluronic 105 | 4 | 50°C | 101±3 | 105±3 |
| GML/LL/PVP | 0 | 65°C | 99±3 | 101±3 |
| GML/LL/PVP | 1 | 65°C | 93±6 | 97±6 |
| GML/LL/PVP | 2 | 65°C | 91±5 | 95±5 |
| GML/LL/PVP | 4 | 65°C | 95±3 | 98±3 |

Each data point represents the mean ± relative standard deviation of three individual samples taken from three separate vials.

**[00104]** Results, presented in the following table, demonstrate that these formulations were able to maintain the stability of the hGH in each case. In each case, at least 70% hGH was retained.

**[00105]** TABLE 8

RECOVERY OF hGH FROM NONAQUEOUS SUSPENSIONS

| Vehicle | %LS by RP-HPLC | %LS by Size-exclusion HPLC |
|---|---|---|
| PVP/PEG 400 | 99±3% | 88±6% |
| GML/LL/PVP | 99±2% | 92±2% |
| Pluronic 105 | 89±7% | 87±7% |

Each data point represents the mean ± relative standard deviation of three individual samples taken from three separate vials.

%LS or % label strength = (measured protein content + theoretical protein content) x 100%

EXAMPLE 6

A. Preparation by Spray Drying

**[00106]** GLP-1 (Polypeptide Laboratories, Wofenbuttel, Germany) was obtained as an acetate salt and was lyophilized. The lyophilized GLP-1 was dissolved in purified water at 19.9 mg/ml and spray dried using a Yamato mini-spray dryer. The spray drying parameters were: 120°C inlet temperature, 90°C outlet temperature, solution flow rate 3.3-5.3 ml/min. Powder was collected in a collection vessel through a cyclone trap. All handling of the ~~spray dried~~ spray-dried powder took place in a dry box evacuated with nitrogen (% RH: 1-4%).

B. Preparation of GLP-1 Formulation

**[00107]** A portion of the ~~single phase~~single-phase viscous vehicle was weighed and heated to 60°C. GLP-1 (Polypeptide Laboratories, Wolfenbuttel, Germany) was added 27% w/w to the vehicle and mixed for 15 minutes. The mixing was completed under vacuum to remove air bubbles.

**[00108]** The resulting suspension was dissolved in 10 ml of release rate buffer and analyzed by size exclusion and reverse-phase chromatography.

C. Analysis of GLP-1 Formulations

**[00109]** The reverse-phase HPLC method consisted of a C-8 5 $\mu$, 4.6 x 250 mm analytical column (Higgins Analytical, Mountain View, CA) with detection at 210 nm. A step gradient method from 25% B to 80% B at 1 ml/~~min~~ min. was as follows: 0-5 ~~min~~ min. at 25% B, 5-30 ~~min~~ min. at 25-50% B, 30-35 ~~min~~ min. at 50-80% B. Mobile phase A was 0.1% TFA in water and mobile phase B was 0.1% TFA in acetonitrile. The formulations were found to be stable for 6 months.

**[00110]** The size exclusion chromatography method consisted of a Pharmacia FPLC HR 10/30 column at a flow rate of 0.5 ml/min. An isocratic method was employed, where the mobile phase was 100 mM ammonium phosphate, 200 mM sodium chloride, pH 2.0, and peptide was ~~deteted~~ detected at 210 nm. The formulations were found to be stable for 6 months.

D. Preparation of Reservoirs

**[00111]** Titanium reservoir systems of implantable drug delivery devices (as disclosed in U.S. Patent Application Serial No. 08/595,761, incorporated herein by reference) were each assembled with an osmotic engine, piston, and ~~rate controlling~~ rate-controlling membrane. The reservoirs were filled with the appropriate amount of viscous vehicle formulation and capped with a flow plug. The systems were placed in a water bath at ~~37°C,~~ 37° C and allowed to release formulation for an extended period of time. Released material was sampled twice per week. Assays for released material were completed using reverse phase HPLC. The resulting concentrations of beneficial agent for each system were converted to release amount per day. The beneficial agent was found to have a zero order release from the implantable drug delivery device.

**[00112]** Modification of the above-described modes of carrying out various embodiments of this invention will be apparent to those of skill in the art following the teachings of this invention as set forth herein. The examples described above are not limiting, but are merely exemplary of this invention, the scope of which is defined by the following claims.

## ~~ABSTRACT~~ABSTRACT OF THE DISCLOSURE

This invention relates to stable ~~non-aqueous~~nonaqueous ~~single phase~~single-phase viscous vehicles and to formulations utilizing such vehicles. The formulations comprise at least one beneficial agent uniformly suspended in the vehicle. The formulation is capable of being stored at temperatures ranging from cold to body temperature for long periods of time. The formulations are capable of being uniformly delivered from drug delivery systems at an exit shear rate of between about 1 ~~to~~ and 1 x $10^{-7}$ reciprocal second.

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